Effects of Habitat Degradation and Fragmentation on the Genetic Population Structure of Phytophagous Beetles in an African Rainforest

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Zusammenfassung

Im Rahmen der vorliegenden Arbeit wurde die genetische Populationsstruktur zweier Käferarten in einem tropischen Regenwald im Westen Kenias untersucht. Über einen Zeitraum von zwei Jahren (Sept. 2001 – Juli 2003) wurden Proben aus unterschiedlichen Gebieten des Waldes gesammelt. Insgesamt konnten 642 Individuen des flugunfähigen Rüsselkäfer *Amphitmetus transversus* (Coleoptera, Curculionidae) sowie 148 Individuen des vagilen Blattkäfers *Monolepta vincta* (Coleoptera, Chrysomelidae) zusammengetragen werden. Für beide Arten wurde ein genetisches Markersystem auf der Grundlage von polymorphen Microsatelliten entwickelt.

Es wurden sechs variable Loci für *A. transversus* sowie neun für *M. vincta* charakterisiert. Das Markersystem von *M. vincta* erwies sich als wesentlich variabler als das von *A. transversus*, was sich in einer höheren Heterozygotie und allelischen Diversität widerspiegelte. Sowohl das Mikrosatellitensystem von *A. transversus*, als auch das von *M. vincta* zeigten in einigen Markern Abweichungen von Hardy-Weinberg Gleichgewicht, was sich in hohen F_{IS} Werten niederschlug. Aufgrund des vorliegenden Musters wurde das Defizit heterozygoter Genotypen durch die Existenz von Nullallelen erklärt, was durch das Auftreten nicht amplifizierter homozygoter Genotypen bestätigt wurde.

Das Mikrosatellitensystem des Blattkäfers *M. vincta* war weniger informativ als das des Rüsselkäfers *A. transversus*. Die genetische Differenzierung von *M. vincta* war gering (F_{ST} = 0,01) und nicht auf die Auswirkungen von geographischer Distanz zwischen den besammelten Populationen zurückzuführen. Es ist wahrscheinlich, dass die hohe Mobilität der Tiere eine genetische Differenzierung in dem kleinräumigen Untersuchungsgebiet verhindert hat. Eine weitergehende Analyse der genetischen Populationsstruktur des Käfers auf Grundlage des hochvariablen Markersystems wurde durch die geringe Probengröße erschwert.

Die Populationen des Rüsselkäfers *A. transversus* zeigten eine moderate genetische Differenzierung (F_{ST} = 0,12), die zum Teil durch die geographische Distanz zwischen den Populationen erklärt wird. Es wurden darüber hinaus drei Gruppen von Populationen gefunden, zwischen denen die genetischen Distanzen maximal sind. Dieses Ergebnis wurde zum einen durch den "Monmonier's maximum difference"-Algorithmus sowie einem Phenogram auf der Grundlage von NEI's genetischer Distanz gestützt. Es wurden mehrere Hypothesen diskutiert, die möglicherweise zur genetische Separierung des südlichen Fragments Kaimosi geführt haben: Die genetischen Auswirkungen der Fragmentierung in Kombination mit einer starken Reduktion der Populationsgröße könnten eine starke genetische Differenzierung einzelner Populationen verursacht haben. Darüber hinaus ist es

möglich, dass Käferpopulationen während Aufforstungen in bestimmte Gegenden eingeschleppt worden sind. Schließlich ist aber auch eine länger zurückliegende historische Separation der Populationen denkbar. Obwohl das Fragment Kaimosi zu den gestörtesten Gebieten des Waldes zählt, ist es fraglich ob die genetische Differenzierung ausschließlich durch die neuzeitlichen Waldveränderungen verursacht worden sind, da ähnlich Effekte in anderen Fragmenten des Waldes nicht gefunden wurden. Es ist wahrscheinlicher, dass eingeschleppte Populationen oder länger zurückliegende historische Gründe das beobachtete Muster verantworten.

Eine weitere genetische Strukturierung von Populationen des Rüsselkäfers trat zwischen dem Nord- und dem Südteil des Kakamega Forest auf. Anthropogene Effekte können hier ausgeschlossen werden, da die Separation innerhalb des zusammenhängenden Waldgebietes auftritt. Es wurde diskutiert, ob das natürliche Flusssystem des Waldes den genetischen Austausch zwischen den Populationsgruppen verhindert hat oder ob die Populationen ökologisch differenziert sind. Beide Hypothesen erfordern weitere Analysen, da auf der Grundlage des vorliegenden Untersuchungsergebnisses eine Abhängigkeit der Differenzierung von der geographischen Distanz nicht ausgeschlossen werden kann. Eine vergleichbare genetische Differenzierung lässt sich jedoch für andere ähnlich entfernte Populationen im Waldgebiet nicht finden, was diese letztgenannte Möglichkeit unwahrscheinlich erscheinen lässt.

Das Ergebnis der vorliegenden Arbeit lässt die Aussage zu, dass anthropogen verursachte Habitatfragmentierung die genetische Populationsstruktur von *A. transversus* beeinflusst. Es konnte gezeigt werden, dass Populationen in kleinen Fragmenten des Kakamega Forests eine geringere allelische Diversität aufweisen als solche im mittelgroßen Fragment und im Hauptwald. Dabei ist eine Abnahme der allelischen Diversität mit der Größe der Fragmente zu beobachten. Weiterhin wurden Tendenzen für eine geringere Heterozygotie in den kleinen Fragmenten beobachtet sowie eine charakteristische Allelfrequenz-verschiebung. Die Ergebnisse wurden durch die Auswirkung von beträchtlichen Waldabholzungen in den Fragmenten mit einer Größe < 200 ha erklärt, die zu einer Reduktion der Populationsgröße der Käfer geführt haben muss. Im Gegensatz dazu wurde in dem mittelgroßen Fragment eine ausgesprochen hohe genetische Diversität gefunden. Dieses Waldgebiet zählt trotz der Fragmentierung zu den ursprünglichsten Gebieten des Kakamega Forest. Eine generell höhere genetische Differenzierung zwischen fragmentierten Habitaten aufgrund von fehlendem Genfluss zwischen isolierten Populationen wurde nicht gefunden.

Die vorliegenden Ergebnisse erlauben den Schluss, dass eine Kombination aus neuzeitlichen, anthropogen verursachten Habitatveränderungen und länger zurückliegenden historischen Prozessen die genetische Populationsstruktur von *A. transversus* geformt hat.

Summary

The present study aimed to analyse the genetic population structure of two beetle species in a tropical rainforest in western Kenya. From September 2001 to July 2003 samples from different parts of the Kakamega Forest have been collected. In total, 624 individuals of *Amphitmetus transversus* (Coleoptera, Curculionidae), an apterous weevil and 148 individuals of *Monolepta vincta* (Coleoptera, Chrysomelidae), a highly active leaf beetle were found. Microsatellite systems were established for each species respectively.

Six polymorphic microsatellite loci for *A. transversus* and nine for *M. vincta* have been developed. The system of *M. vincta* turned out to be much more variable than that of *A. transversus,* which was expressed in a higher heterozygosity and allelic variability. Both the marker system of the weevil and the leaf beetle showed deficiencies of Hardy-Weinberg-Equilibrium (HWE) at several markers, which was also reflected in high F_{IS}-values. The high heterozygote deficits were explained by the existence of null alleles at the concerning markers, which was also confirmed by the existence of null homozygotes.

The system of *M. vincta* was less informative regarding the population structure of the beetle at the given micro geographical scale. The populations exhibited a weak genetic differentiation (F_{ST} = 0.01) across the geographical scale, which could not be attributed to the geographical distance between sample sites. The result is probably caused by the high mobility of the leaf beetle. A more detailed analysis of the genetic population structure on the given marker system was limited by the number of sampled individuals.

A. transversus was genetically differentiated across the observed range (F_{ST} = 0.12), which was partly explained by an 'isolation by distance' pattern. Furthermore, a separation in three population cluster was revealed, which was confirmed by Monmonier's maximum difference algorithm as well as by a population's phenogram based on NEI's genetic distance. The population of the fragment Kaimosi is maximally differentiated to all other populations. Three explanations of this pattern have been invoked: Fragmentation in combination with an extreme bottleneck, introduced populations during the extensive plantation of the area as well as historically restriction of gene flow have been considered. Although the fragment Kaimosi is the most disturbed area of the Kakamega Forest it is questionable if the large genetic differentiation can be explained by contemporary fragmentation alone, because similar changes were not found for other small fragments. It was concluded that most likely introduced beetle populations or historical causes in combination with the recent fragmentation have led to the genetically isolated constitution of the population.

A second separation of the population was found between the northern and the southern part of the Kakamega Forest. Anthropogenic effects can be ruled out as the pattern occurs in the continuous forest, that was not separated as far back as records extend. Possibly a natural river system prevents gene flow between these population groups, but the hypothesis remains to be tested by an adequate sampling along both sides of the Isiukhu River, which dissects this part of the forest in separated areas. Another consideration invokes the ecological differences that might have led to ecologically distinct population groups which are reflected in genetically differentiation. The sampling was designed for the analysis of fragmentation effects in the first place and did not allow a clear examination of other reasons of genetic differentiation. Geographical distance as a cause could not be precluded, although this seems to be rather unlikely. Comparable geographical distances between other populations of the forest have definitely not led to a similar genetic differentiation.

The results of the present study partly indicate that anthropogenic introduced habitat fragmentation affects the genetic structure of the weevil *A. transversus* as predicted by theory. It has been found that populations of the small fragments in the Kakamega Forest show significant lower genetic diversity than populations of the moderate fragment as well as of the continuous forest, whereas the genetic variability of the populations decreases with the fragment size as measured by allelic richness. A mode shift and tendencies of a decrease in heterozygosity support the outcome. The results were explained by the effect of large deforestation at fragments of size smaller than 200 ha, which must have been resulted in a bottleneck of *A. transversus* populations in these areas. In contradiction, the moderate fragment of size larger than 400 ha did not reveal changes of genetic diversity in similar direction. The fragment, that, despite of its geographic isolation, belongs to the most conserved areas of the Kakamega Forest, even reveals the highest genetic variability of all analysed sites. It was not found that fragmentation of habitat generally leads to an increase of genetic differentiation due to the restriction of migration and hence gene flow between isolated sites.

The genetic population structure of *A. transversus* in the Kakamega Forest appears to be influenced by both, contemporary and historical habitat changes. The study is evidence that anthropogenic fragmentation has always to be interpreted in the context of long-term population history.

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The gates of Hell are open night and day
Smooth the descent, and easy is the way:
But, to return, and view the cheerful skies;
in this, the task and mighty labor lies.
Virgil

1. Introduction

1.1. General Background

Human impact on natural resources has immensely increased during the last decades and led to an enormous crisis concerning the threat to biodiversity. On the Earth Summit held in Rio de Janeiro 1992 the ubiquitous decrease of biodiversity became a central concern in policy for the first time. The worldwide awareness of the problem has grown dramatically and the practice of biological conservation as part of national policy has been adopted by a lot of countries. There is wide agreement that the most invasive threat to biodiversity is habitat loss and degradation (WILCOVE *et al.* 1998), which is accompanied by an extensive fragmentation of the available area into patches that are isolated from each other by less suitable landscape elements, such as agricultural areas, settlements and roads. This problem affects in particular the highly species diverse tropical rainforests, because of ongoing massive deforestation, land use change and urbanization (WHITMORE 1997). In Africa, 7,025,020 km² of forest in 1990 have been reduced to 6,498,660 km² in 2000. That is equivalent to a decrease in forest cover of approximately 52,620 km² per year. The extent of fragmentation is even much greater. In the Brazilian Amazon, for example, the area of forest that is now fragmented to remnants of less than 100 km² is more than 150 % higher than the area that has actually been deforested (FAO 2003). Beginning with the impoverishment of genetic resources, continued by the extinction of single species and disconnection of communities, the consequences of human activities inevitably lead to the destruction of whole ecosystems.

Traditionally, the term biodiversity is defined by those inter-linked levels of biological organization – the ecosystem, the species and the gene. The genes, as the basic units of heredity, form the most inclusive level of biological diversity. The gene pool of a population constitutes the fundamental unit on which evolutionary forces act, while environmental changes drive those effects and are expected to leave their footprints in the genetic diversity of a species. Habitat loss and fragmentation may reduce population sizes and change the spatial distribution of remaining subpopulations by confining them to remnant patches. The patchy distribution of fragmented forest habitats prevents gene flow between populations and restricts their expansion to new habitats (TEMPLETON *et al*. 1990). Reduced population size

and the isolation of subpopulations in turn, may result in increased genetic drift and inbreeding, leading to a loss of genetic variability within and an increase of genetic differentiation among populations (WRIGHT 1931, NEI 1975). A loss of genetic variability may have important consequences for the long term viability of populations, due to the decreasing ability of populations in responding to environmental changes (SELANDER 1983). Without variation between individuals, a population cannot adapt to changing environments and is vulnerable to new predators, diseases, parasites, climatic conditions, competitors or changing food supplies (LACY 1987). Parameters which determine the magnitude of effects resulting from a decline in population size and density are given by the extent to which population sizes and migration rates are reduced as well as the time since isolation (SLATKIN 1987).

Effects of anthropogenic fragmentation cannot be interpreted without the context of long term population history (TEMPLETON *et al*. 1990). Regarding the genetic consequences of contemporary habitat fragmentation, the assumption has to be implicated that the non-fragmented area represent the pre-fragmentation state that can be directly compared to the fragmented area. However, previous historical processes rather than human induced changes may be major contributors to extant genetic patterns in control sites and even fragments. Such processes may cover the contemporary effects of habitat fragmentation and have to be taken into consideration in the examination of human induced changes (BERMINGHAM & AVISE 1986, CUNNINGHAM & MORITZ 1998).

It is expected that hyper variable markers like microsatellites are useful in analysing the effects of contemporary habitat fragmentation. The comparable high polymorphism offers the possibility to examine population genetic studies on a considerable small geographical scale. Furthermore, it is assumed that their high mutation rates of approximately 10^{-3} events per locus per replication (WEBER & WONG 1993, JARNE & LAGODA 1996) quickly restores variation caused by historical events, and more recent population fluctuations can be detected. Hence, those molecular tools provide an effective mean of quantifying the effects of landscape structure on the geographical pattern of genetic variation.

1.2. State of Research

Habitat changes and destruction as causes of geographical separation and bottleneck effects have been reported in many plant and animal studies of temperate climates (e.g.

LEBERG 1991, LINDENMEYER & PEAKALL 2000, STOW *et al*. 2001, BELLINGER *et al*. 2003, CULLEY & GRUBB 2003, MILLER & WAITS 2003, MARTÍNEZ-CRUZ *et al.* 2004), including also several studies on invertebrates and especially beetles (DESENDER *et al*. 1998, KEYGHOBADI *et al*. 1999, NIEMELÄ 2001, MONAGHAN *et al*. 2002, BRITTEN *et al*. 2003, BROUAT *et al*. 2003, 2004, KELLER *et al*. 2004, KRAUSS *et al*. 2004, TAKAMI *et al.* 2004). However, comparable genetic studies on insects in tropical ecosystems are missing, although the group contains the vast majority of species diversity. Almost a million insect species has been recognized (OREN 2004) in comparison to ca. 1.5 million described taxa (BLAXTER 2004).

Tropical rainforests inhabit the most species rich terrestrial animal communities, while habitat fragmentation is one of the most serious environmental threats confronting the plant and animal species of these biomes. The research activity on the genetic and demographic effects of human impact has mainly focussed on a variety of vertebrates or plants in tropical habitats (CUNNINGHAM & MORITZ 1998, MORDEN & LOEFFLER 1999, SRIKWAN & WOODRUFF 2000, SUMNER *et al*. 2004, GALBUSERA *et al*. 2004). The comparatively small numbers of individuals in populations of vertebrate species are supposed to be a reason for these animal groups to show a conspicuous effect of population bottlenecks due to contemporary habitat destruction. On the other hand, long generation times might reduce the rate at which reductions in population sizes are reflected in the genetic data. The relatively small spatial scale and the rapid generation time of most invertebrates makes them particularly useful in testing hypothesis of demographic and genetic impact of fragmentation (CLARKE 2000).

BARBOSA & MARQUET (2002) as well as DIDHAM (1997) reported that environmental changes, caused by fragmentation, affect invertebrate and even beetle assemblages in tropical systems, while, up to now, genetic effects have been recorded for temperate climate only. Furthermore, it has to be recognized that the diversity of tropical forests is predominantly based on insects. About 40 % of all species belong to the beetles (Coleoptera) as the most diverse group (e.g. HAMMOND 1990, 1994, WAGNER 2000). Particularly Staphylinidae, Curculionidae and Chrysomelidae along with Hymenoptera, Lepidoptera and Araneae are the most specious taxa in the canopy (BASSET 2001). Hence, phytophagous insects are one of the dominat insect groups in tropical forests with a key position in the ecological food web. Some species show close food plant relationships, while most of them are polyphagous, and their occurrence is mainly affected by microclimatic habitat conditions (WAGNER 2000). Thus, change in forest structure, size as well as fragmentation is expected to constitute crucial factors for their occurrence and organization. As there is poor knowledge about these topics

concerning phytophagous beetle species in the tropics, an investigation of the population organization on the genetic level is useful in order to exhibit the organization of genetic diversity and differentiation.

1.3. Aim of the Present Study

Up to now, there is hardly any knowledge about the population structure of phytophagous beetles in tropical biomes. A central aspect of the present study was the examination of the genetic population structure of two beetle species in a tropical rainforest with special regard to the effects of contemporary deforestation and habitat degradation on the level of genetic diversity. It should be evaluated if phytophagous beetles, that show comparably high population densities, exhibit a changing population structure due to human impact.

The study was carried out at the Kakamega Forest, a highly fragmented rainforest in western Kenya, which represents a remnant of the guineo-congolian rainforest complex. The total area is of relative small size providing a large part of continuous forest and several surrounding fragments of various age and size (BROOKS 1999, MITCHELL 2004). The conditions of the Kakamega Forest are well suited for an examination of historical and anthropogenic influences on the population genetic structure due to its well-known history.

The statistical analysis of population genetic data depends on a representative sample size. While invertebrates generally show high population densities in temperate climate, these characteristics are not similarly found in the tropics. The beetle fauna of tropical rainforests is characterized by high species richness, but low population densities due to many rare species (WAGNER 2000). Therefore, the sampling of an adequate number of individuals was expected to be difficult and the investigated insects were primarily chosen by their relatively high abundance and their restriction to wet forest biomes. Individuals of two species, an apterous weevil (*Amphitmetus transversus*) (Curculionidae) and a highly active representative of the leaf beetles (*Monolepta vincta*) (Chrysomelidae) have been sampled at sites across the Kakamega Forest during several excursions within the years 2001–2003.

In this context, the objective of the study was to determine the genetic structure and genetic diversity of *Amphitmetus transversus* and *Monolepta vincta* on the given micro geographical scale, based on the variation of polymorphic microsatellite markers. The study focussed on the following questions:

- Are the populations of *A. transversus* and *M. vincta* structured significantly on the investigated area of the Kakamega Forest?
- Does the genetic differentiation between populations depends on the limited migration between separated habitats?
- Do natural or anthropogenic influences cause the observed pattern?
- Does the degradation and fragmentation of the forest affect the genetic diversity of the beetles?
- Are phytophagous insects in tropical forests a useful indicator of forest change and fragmentation on the population genetic level?

Uwaingila mu mushintu, tomfwa nswanswa.
One who enters the forest does not listen to the breaking of the twigs in the brush.
African Proverb

2. Study Site

2.1. The Kakamega Forest

2.1.1. Location and Characterization

The Kakamega Forest is located in the Shinyalu Division of the Kakamega District in the Western Province of Kenya between the latitudes 0°10' and 0°21'N and longitudes 34°47' and 34°58'E. It is situated in the Lake Victoria basin on the most eastern edge of the Central African rainforest area about 40 km north of Kisumu and just east of the Nandi Escarpment that forms the edge of the central highlands (Fig. 1).

Fig. 1: Map of Africa (left) and Kenya (right). The location of Kakamega Forest is indicated with a red point on the right map (Reference: Microsoft Encarta Weltatlas 2001).

The Kakamega Forest is the only rainforest in Kenya and the flora and fauna is influenced both by guineo-congolian as well as by afromontane elements. The forest receives between 1.500–2.300 mm of precipitation per year. Most of this rain falls between April and November with a short dry season from December to March. Average monthly maximum temperature ranges from 18 to 29 °C (KOKWARO 1988) and the average monthly minimum

from 4 to 21 °C (MURIUKI & TSINGALIA 1990). Its altitude above sea level varies between 1400–1700 m and the Kakamega Forest can be classified as a "mid altitude tropical rainforest" (KOKWARO 1988). The Reserve covers an area of about 240 km² and consists of a mosaic like structure of different vegetation types (Fig. 2). Nearly half of it constitutes an area of continuous forest (10100 ha) (BLACKETT 1994). Factors, which are shaping the heterogeneous aspect of the forest are biotic (habitat structure), abiotic (climatic differences, soil structure) as well as historic (see history of forestation) and especially anthropogenic (ALTHOF *et al.* 2003, MITCHELL 2004). The forest is drained by a number of rivers and streams. Two major rivers pass the forest from East to West, each with several tributaries; the Yala River crosses the southern part of the forest, while the Isiukhu River flows through the northern part (Fig. 7).

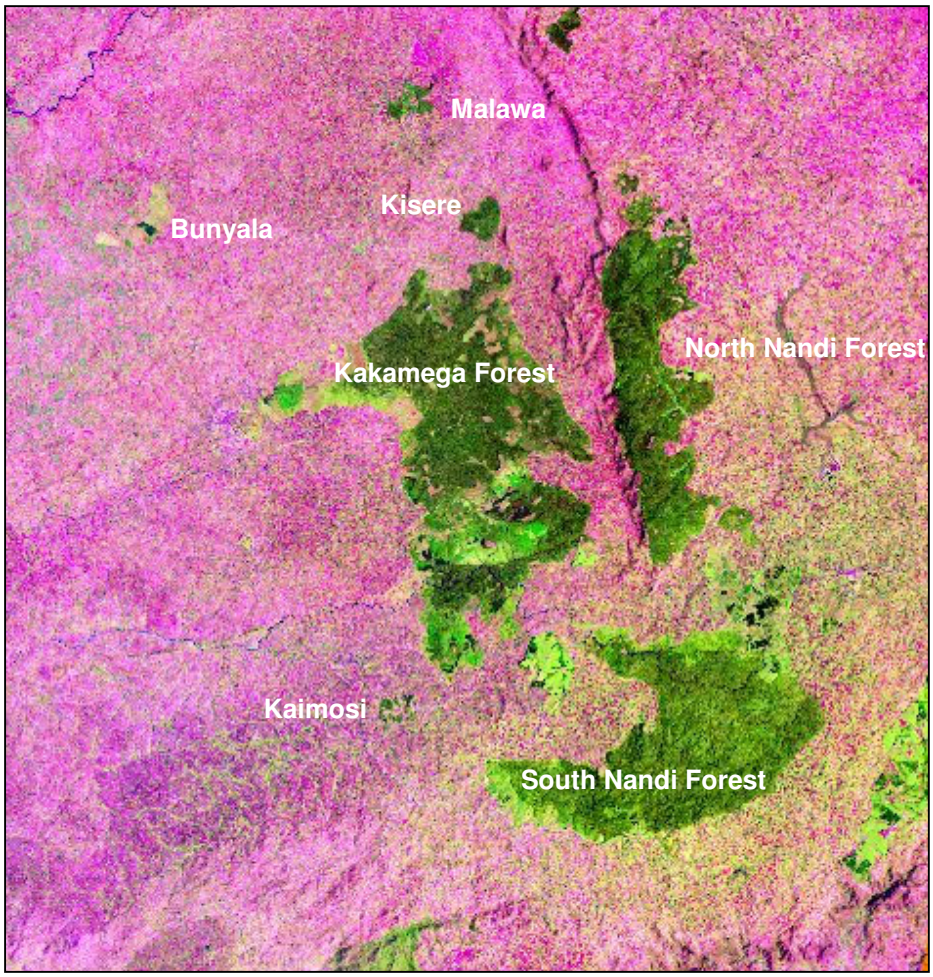

Fig. 2: Satellite image (channel 5 of Landsat 7 ETM+, 05 Feb 2001) of the area around Kakamega. Forested parts are indicated green including Kakamega Forest and adjacent fragments as well as South and East Nandi Forest (Reference: BIOTA E02).

With a population density of more than 175 people per km², the Kakamega district belongs to Africa's most densely populated regions (TSINGALIA 1988, WASS 1995) . OYUGI

(1996) claims a population increment of 2.8 % per year. Therefore, the surrounding countryside is used intensively for growing sugar cane, maize and tea, and the forest itself is under increasing pressure. It is affected by timber cutters, charcoal burners as well as firewood collectors (KOKWARO 1988, MITCHELL 2004). A disturbance gradient from primary like forest to secondary forests as well as completely degraded areas can be recorded, while the main part of the Kakamega Forest consists of secondary forest (ALTHOF *et al.* 2003). The forest has some fragments in its vicinity, which differ in distance, size and age. Forested areas, which are totally separated from the main part of the forest and constitute forest islands surrounded by agricultural landscape are Malawa, Kisere, Bunyala and Kaimosi. Malawa, Kisere and Bunyala are located in the periphery of the northern part of the Kakamega Forest, while Kaimosi represents the most southern part. The fragments exhibit different degrees of degradation. Generally, a gradient of increasing disturbance ranges from Kisere to Malawa, to Kaimosi and at least to Bunyala (ALTHOF *et al*. 2003).

The North Nandi Forest is located at an altitude between 1700 and 2130 m above sea level and stretches across the East Nandi Escarpment. Due to its higher elevation, the forest vegetation differs from that of the Kakamega Forest and a higher amount of montane species are found. The South Nandi Forest is located at a similar altitude from 1700 to 2000 m and species composition is similar to that of North Nandi Forest. Formerly, it was connected with the southern part of the Kakamega Forest (MITCHELL 2004).

2.1.2. The Early History of Kakamega Forest

During the last 2.3 Myr Pleistocene climatic fluctuations caused considerable changes in the vegetation and in particular the central and north tropical African forest distribution. It has been suggested that there have been 21 glacials or near glacial periods since that time (VAN DONK 1976). The most recent world glacial maximum ended approximately 12000 yr BP ago. This last glaciation has led to a period of dry and cold climate and hence caused the last significant contradiction of the pan-african forest zone. Evidence for the mentioned effects on the east African climate and the forest distribution comes from geomorphological features as well as fossil findings, like pollen analyses or plant and animal macrofossils (overview in LOVETT & WASSER 1993). From the beginning interglacial the African forests expanded again (HAMILTON 1982). Pollen sequences show that between 12000 and 10000 BP lowland forests increased in Uganda (HAMILTON 1981, 1984) and MITCHELL (2004) imagines that at the Kakamega region the same process appeared. HAMILTON (1982) analysed the factors affecting

seed dispersal and colonizing ability with regard to the species representation in Uganda and specifically the Kakamega Forest. He suggests that the montane tree species of the Kakamega Forest probably derived from populations that survived the arid phase in montane areas of Kenya such as Mount Elgon and Cherangani Hills. The expansion of the vegetation did not proceed in a single full fronted advance from the west, but by colonising species establishing themselves as islands of woodland that later matured and resulted in dense forests (MITCHELL 2004). From this view it was discussed that the fragments Malawa, Kisere and Bunyala never have been fully joined to the main part of the Kakamega Forest after the last glacial. Additionally, early pastoralists who settled in the area, regularly set fires in the grasslands that may have prevented these forests from joining each other via more than a network of forested rivers (MITCHELL 2004). However, following the KIFCON opinion, the Kakamega Forest had a homogenous structure and composition until human pressure was exerted on the forest (MUTANGAH *et al*. 1992).

2.1.3. The Contemporary History of Kakamega Forest

With the beginning of colonization in the early 20th century, the documentation of the forest's history became more reliable and the impact of human activities more evident. The man-made fragmentation history began in the late 19th century. Assuming a continuous forest cover including all fragments, BROOKS (1999) stated that the total forest block must have amounted almost 25,000 ha. Up to this point the whole area of the Kakamega Forest was already influenced by anthropogenic activities such as repeated burning of grasslands by pastoral tribes and African shifting farmers (KOKWARO 1988). The construction of the Uganda Railway between 1895 and 1901/2 allowed the British colonizers easy access to the western part of Kenya (MITCHELL 2004) and was the beginning of a major human impact on the shape of the Kakamega Forest. The need of fuel for the wood-burning Mombasa-Kisumu railway led to the first up-country forest reservation and initiated the establishment of eucalyptus plantations (KOKWARO 1988).

In 1931 gold was discovered in the forest. This attracted hundreds of European prospectors and led to the foundation of companies like "Tanganyika Concession Ltd" or "Rostermann Goldmine", who employed several thousand local people (MITCHELL 2004). Concessions for prospecting were also located in the forest area, of which the area around Isecheno is one of the most affected as well as the areas along the Isiukhu River and the main Isecheno-Ikuywa road (Fig. 7). The gold rush climaxed in the middle of the 1930's and was

damped down with the beginning of the Second World War. In addition to the general impact of the gold mines to parts of the forest, they caused the opening of the forest to official and commercial scale logging.

The first logging concessions were given out in 1933 and mines maintained a fluctuating demand for pit-props until the late 1940's (MITCHELL 2004). With the advent of the sawmills the industrial exploitation of the forest began on a grand scale. They operated in Kakamega for almost 50 years and affected the forest both by clear cutting and selective felling. In the first case, trees of certain species and diameter were cut out and the remaining trees were turned into charcoal and firewood for sale, while in the second case only certain species and diameters of trees were removed. These procedures were practised in the Kakamega Forest between 1933 and at least 1956. No forest appears to have been clear cut from the late 1950's to about 1970 when clear felling was re-introduced. It was supposed to be banned again from 1974/75 on, but considerable areas of the forest have disappeared after this time. After 1975 much of the remaining indigenous forest was subject to selective logging for a wide range of species for both timber and plywood industries (MITCHELL 2004).

Additionally, so called pit-sawyers were officially licensed by the Forest Department to cut timber from areas already exploited by sawmills. The sawyers' job was to remove the timber trees that had been missed or omitted as uneconomical for the sawmills to take. Lastly, charcoal burners were normally employed to clear the forest in preparation for replanting after sawmills and the pit-sawyers had taken all the timber in a concession area. By this procedure the indigenous forest cover of the Kakamega Forest has been reduced from 23,785 ha in 1933 to 13,990 ha in about 1990 (MUTANGAH 1992). Most of this clear felling was done in the southern half of the forest and in the western arm near of Kakamega town. Clear felling has not only destroyed nearly half of the forest, but resulted also in a degree of islandisation. The Yala and Ikuywa areas have become virtually separated from the forest to the north and are connected to each other by only a narrow strip of forest. The extent and effect of selective logging is less obvious and more difficult to assess than that of forest clear cuts. All of what remains as indigenous forest today, with the exceptions of the Yala River, the Isecheno Nature Reserves and the Kisere National Reserve, appears to have been selectively logged at some time since 1933 (MITCHELL 2004).

Glades and clear-cuts provided the opportunity for the first plantations at Kakamega around 1934, south of Isecheno. These consisted of indigenous trees and were soon followed by monocultures of Eucalyptus. In the 1940's the first conifers have been planted at Isecheno, which represented 1999 ha in 1990 (MITCHELL 2004). Estimates of the total plantation area in

the Kakamega Forest vary between 1700–2400 ha, mainly in the southern part. With the extensive plantation in the above mentioned area, the beginning separation of the southern part of the main forest started in the 1960's (BROOKS 1999). The separated parts Yala and Ikuywa were shaped, which today are still surrounded by plantations and therefore cannot be characterized as real fragments in a narrow sense.

The general history of the northern part of the forest is mainly influenced by growth and expansion into the surrounding grasslands. Regions around Salazar and from Isiukhu Falls to Buyangu Hill must have been grassland in the recent past. These areas were forested a hundred years ago. Although some logging took place in the northern part of the forest it was not as heavy as in the southern area. Since KWS took over the administration of the Kakamega National Reserve in the North, the encroachment was confined abruptly (MITCHELL 2004).

2.1.4. History and Characterization of the Fragments

The Bunyala fragment is located in the north-western direction of the main forest block (Fig. 2). Today only a small remnant is left. The original forest is mainly replaced by plantations. It is the smallest and most destroyed fragment of the Kakamega Forest.

As far back as records extend, the Malawa fragment was already separated from the main forest. For this reason it can be assumed, that Malawa was separated from the main forest not later than 1910. Malawa is nowadays divided by the Kakamega-Eldoret road in a western and an eastern part. According to the different aspects of the Malawa fragment, it is differentiated into at least three sub-parts, which are named differently by the local people. The eastern part is called Shitirira, which means "trembling", because of the great incidence of cattle inside the forest. North of Shitirira the forest was originally known as Mungaha (or Muhoni) which means "fertile" as a result of the black fertile soil here. The area west of the road is known as Malawa as this word means "clean" due to the light understorey (MITCHELL 2004). Intensive logging in this area started in 1940. By 1952 the forest east of the road had already been completely logged and re-planted. Between 1945 and 1957 a policy was devised to allow the regeneration of the most valuable species in the logged areas. The natural regeneration was supplemented by 250 acres of group planting in small internal clearings. One hundred acres were allowed to regenerate naturally without the aid of group planting and this is probably the area west of the road. Malawa has therefore experienced heavy selective

logging. The fragment size of 718 ha at the beginning of the 20th century has been reduced to the existing fragment size of 190 ha (KOKWARO 1988) (Table 1).

The Kisere forest fragment was first documented in a photograph of 1948, where it seems to have the same size as today (400 ha) (Table 1). BROOKS (1999) estimated that Kisere was isolated around 1933, when the forest was first gazetted, but recent investigations draw another picture. According to MITCHELL (2004), Kisere Forest was already separated and of nearly the same size as today in 1913/16, which can be verified by maps of those days. TSINGALIA (1988) stated that Kisere was never connected to the Kakamega Forest in the 20th century by anything more than the assumed connection along the Isiukhu and Nandamaywa Rivers, which surround this part of the forest (Fig. 7). This forest has always been protected from large scale exploitation of the major sawmills. Kisere's inaccessibility, cut off from Kakamega town by the Isiukhu and Nandamanywa Rivers, ensured that no sawmill could operate there without great difficulty (MITCHELL 2004). This is consequently one reason for a relative lack of human disturbance in the fragment.

The southern fragment at Kaimosi is one of the oldest well known collection localities in Kenya (VAN SOMEREN 1920) and was always under the control of the local population. The Christian Kaimosi station was set up in 1902 and the land was never under the control of the government again (MITCHELL 2004). On a map of 1913/16 the forest seems to be connected with the South Nandi Forest as well as with the Kakamega Forest, while the map of 1959 shows fragments of presumably residual forest less than half a kilometer from the south east corner of the Kaimosi mission. Furthermore, the present forest remnant is placed outside of the original forest cover of 1913/16 (MITCHELL 2004) and is most likely a plantation. In 1965 the local Quaker church took over the mission, and pit sawing was started by the local people that continued until present. The area south of the mission was totally clear-felled and the

Table 1: Size of Continuous Forest and Fragments of the Kakamega Forest obtained from satellite image 05 Feb 2001 Landsat 7 ETM+ (Reference: BIOTA E02).

Part of the Forest	Size [ha]
Continuous Forest	10793
Kisere	420
Malawa North & East	113
Malawa West	77
Kaimosi	65

eastern part was severely affected by selective logging (MITCHELL 2004). The forest at the Kaimosi mission is a fragment, which definitely was once attached to the main area of the Kakamega/South Nandi Forest. However, it is difficult to assess the date of its isolation from the South Nandi Forest more accurately than between 1913 and 1959 when the forest to the south-east was being gradually eroded (MITCHELL 2004). The today remaining forest covers an area of approximately 65 ha (Table 1).

2.1.5. The Administrative History of the Kakamega Forest

The Kakamega Forest was first gazetted as a trust forest in 1933 when the Forest Department took over the management. Two small Nature Reserves, Yala and Isecheno were established within the forest reserve in 1967 (BLACKETT 1994), but partial exploitation were still allowed (ANALO 2003). In 1986, nearly 4000 ha of the northern part of the forest together with the adjacent Kisere Forest were gazetted as the Kakamega Forest National Reserve. These parts are now administrated by the Kenya Wildlife Service (KWS). In 1984, a presidential directive banned the conversion of indigenous forest to plantation and another in 1988 banned the cutting of indigenous forest trees. In 1991, a memorandum of understanding was drawn up between the Forest Department and the KWS, who are now supposed to co-operate. However, many of the rules are not strictly enforced by the Forest Department, while, in contrast, the KWS very strictly prohibits local use of the forest in the National Reserves of Kisere and Buyangu (BLEHER *et al*. 2004, MITCHELL 2004).

Per aspera ad astra
Latin proverb

3. Material and Methods

3.1. Species

The diversity of tropical forests is predominantly based on insects. About 40 % of all insects and 30 % of all animals belong to the beetles (Coleoptera) as the most specious group (e.g. HAMMOND 1990, 1994, WAGNER 2000). Beetles are found in almost every available terrestrial and freshwater habitat. The Curculionidae with approximately 75.600 nominated species and the Chrysomelidae with approximately 35.000 nominated species belong to the largest groups of the beetles (KAESTNER 2003). The important ecological role of the mainly phytophagous species group can not be doubted. The weevils are highly variable in form while they are characterized by the elongation of the anterior part of the head. Often they are robust and heavily sclerotised and clothed with scales or bristles. The most groups are phytophagous, while the larvae are almost always feeding internally in plant tissue or roots (BASSET 2001). Leaf beetles are similar to weevils in having pseudotetramerous tarsi. They differ from weevils, however, in having well-developed mouth parts but no rostrum. Generally, leaf beetles and their larvae have extremely varied body forms. All representatives of the leaf beetles are phytophagous, the adults feeding externally and the larvae externally or internally on a wide variety of higher plant tissues comprising roots, leaves, flowers or pollen (LAWRENCE & BRITTON 1991).

Two beetle species, a representative of the weevils (Curculionidae) and one of the leaf beetles (Chrysomelidae) were chosen to examine the effects of the long term structuring and the contemporary habitat fragmentation of the Kakamega Forest on the population genetic level.

3.1.1. *Amphitmetus transversus* (Kolbe 1897) (Coleoptera, Curculionidae)

Amphitmetus transversus (KOLBE 1897) (Coleoptera, Curculionidae) is a weevil of black colour with yellow or green scales (Fig. 3). Coxa, femur and tibia are reddish, while the tarsi are black. The elytra are convex, with ten longitudinal stripes and characterized by two yellow scaled dimples. The geniculate antennae are covered with black bristles. The relatively

short, stout rostrum contains a longitudinal furrow. *A. transversus* is distributed across the guineo-congolian rain forest complex of Africa. The weevil is apterous and is therefore characterized by a low mobility. It feeds on a wide variety of different vascular plants and is not specialized in this way. The species is abundant inside the forest areas and can be found in all parts of the Kakamega Forest, including fragmented and degraded areas. As field studies show, it is restricted to shady forest sites.

Fig. 3: *Amphitmetus transversus* (KOLBE 1897) (Coleoptera, Curculionidae), Kakamega Forest

3.1.2. *Monolepta vincta* Gerstaecker, 1871 (Coleoptera, Chrysomelidae, Galerucinae)

About 160 nominal *Monolepta* species are described worldwide mainly from tropical regions, including 160 from Africa (WAGNER 2003). *Monolepta vincta* Gerstaecker, 1871 (Coleoptera, Chrysomelidae, Galerucinae) is the most abundant afrotropical *Monolepta*

Fig. 4: *Monolepta vincta* Gerstaecker, 1871 (Coleoptera, Chrysomelidae) (Photo: Th. Wagner)

species. It is highly polymorphic in coloration, but characterized by yellow to yellowish-red elytra and two transverse black elytral bands at the base and in the apical (Fig. 4).

The head and prothrorax are yellow to red, while the abdomen is mostly yellow. The antennae are pale yellow, only the last antennomere or the most apical parts of the tenth antennomere are coloured brownish or black (WAGNER 2004). The species is widely distributed in lowland and montane areas of eastern and western Africa where it occurs mainly in wet savannas and tropical forests. In comparison to *A. transversus, M. vincta* is able to fly and shows a highly active behaviour.

3.2. Sampling

The statistical analysis of population genetic data needs a representative sampling of individuals per population. Therefore, a method was needed that allows a quantitative sampling of the concerning species. A lot of the available sampling procedures (see BASSET *et al*. 1997) focus primarily on the record and analysis of arthropod or beetle communities (e.g. branch clipping, Gassing, Insecticide Knockdown) and are less suitable for the collection of single species. Furthermore, the DNA of the beetles had to be preserved in 99.98 % Ethanol directly after sampling to allow a successful molecular analysis. The usage of traps (e.g. Malaise traps or composite flight-interception traps) often do not allow a preservation of the specimens directly after the collection, especially in the tropics where alcohol of the needed concentration evaporates immediately due to the high temperatures.

However, simple hand-sampling methods have turned out to be the most successful method, since it allows a fast and easy inspection of smaller canopies and shrubs as well as a sampling and preservation of single species, since the collection was visual inspected immediately. Hence, probes were taken primarily by beating to achieve an acceptable number of individuals per plot. Furthermore, several specimens were collected during the canopy fogging experiments of a related project.

3.2.1. Canopy-Fogging

Eight conspecific trees (*Teclea nobilis* and *Heinsenia diervilleoides*), 8–13 m in height, at five sites of the Kakamega Forest were each fogged from the ground for about 4

minutes with natural pyrethrum (1.5 % active ingredient) as insecizide, using a Swingfog SN-50. Falling arthropods were collected on 16 1-m² sheets hung near the trunk under the canopy (Fig. 5). All individuals which fell during a drop time of 1½ hour were collected and preserved in alcohol. Specimens of either of the two species, *Amphitmetus transversus* and *Monolepta vincta*, were sorted later in order to use them for population genetic analyses.

Fig. 5: Canopy Fogging of *T. nobilis* at the Colobus Trail in the Kakamega Forest.

3.2.2. Hand-Sampling

The specimens were collected by beating. During the procedure a beating tray is hold under a few branches of smaller canopies or shrubs, which are then struck with a stick (Fig. 6). The concerned species were collected with aspirators and afterwards conserved in tubes that were filled with 99.98 % ethanol. The geographic position of the individual was recorded.

Fig. 6: Beating at the sample site Busumbuli II in the Kakamega Forest

3.3. Sample Sites

Sample areas were located at 30 sites across the Kakamega Forest (Fig. 7), which were situated within the fragments and within the continuous forest. It was attempted to select as many sites as possible to cover the total forest area. Areas of hand-collecting were chosen by

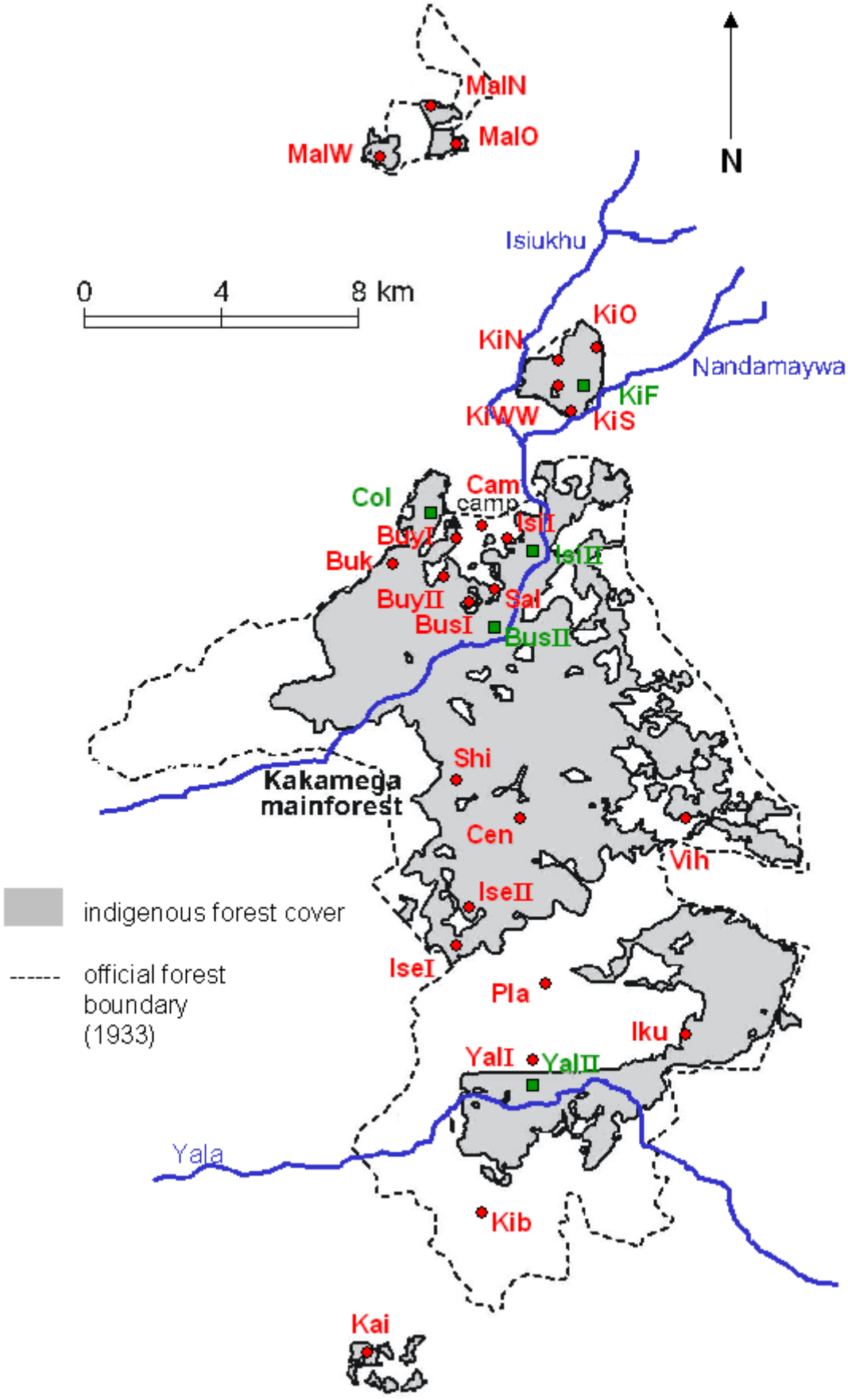

Fig. 7: Scheme of the Kakamega Forest with sample sites of beating (points) and canopy-fogging (squares) (Map: Schaab; E02/GIS-FE; main rivers added).

their accessibility through pathways and the ability to reach smaller canopies and shrubs. The canopy-fogging took place at five different locations. Eight specimens of *Teclea nobilis* (Rutaceae) at the locations "Colobus Trail", "Busumbuli II", "Isiukhu II" and "Yala II" as well as *Heinsenia diervilleoides* (Rubiaceae) at the location "Kisere Fogging" and "Colobus" were fogged.

Individuals of the weevil *Amphitmetus transversus* were collected at 29 sites, but some locations did not provide an adequate number of individuals or the amplification of DNA failed for too many genotypes. These sites were consequently not included in the analyses (Table 2). The sample sites had an extent of 0.1 km² on average. Distances between plots ranged from 0.5 km between Busumbuli I and II to 36.98 km between Malawa North and Kaimosi. Pairwise distances between plots are given in the matrix of geographical distances (Appendix Table A5 & A10). *Monolepta vincta* could be collected at seven locations only. The sampling took place at different times over a period of two years: Sept/Oct 2001, Jan 2002, Sept/Oct 2002, Jan 2002 and Jun/Jul 2003.

Table 2: Sample sites and respective site code of *Amphitmetus transversus* and *Monolepta vincta* in the Kakamega Forest, Kenya. N = number of sampled individuals over two years (Sept 2001–July 2003). Bold type = populations, which were included in further analyses.

Sample Site	Site Code	*Amphitmetus transversus* (N)	*Monolepta vincta* (N)
Continuous Forest:			
Colobus	Col	**54**	**28**
Busumbuli II	BusII	15	**22**
Busumbuli I	BusI	**30**	-
Salazar	Sal	**19**	-
Campsite	Cam	**44**	**18**
Isiukhu I	IsiI	**33**	-
Isiukhu II	IsiII	9	**41**
Buyangu I	BuyI	**35**	-
Buyangu II	BuyII	9	-
Bukhyawa	Buk	**28**	-
Shiamololi	Shi	3	-
Yala I	YalI	**34**	-
Yala II	YalII	19	**22**
Ikuywa	Iku	**23**	-
Isecheno I	IseI	**22**	**12**
Isecheno II	IseII	9	-
Center	Cen	1	-
Plantations	Pla	10	-
Vihiga	Vih	**14**	-
Kibiri	Kib	**23**	-
Fragments:			
Bunyala	Bun	-	-
Malawa West (Malawa)	MalW	**24**	5
Malawa East (Shitirira)	MalE	**24**	-
Malawa North (Mungaha)	MalN	**19**	-
Kisere North	KiN	**22**	-
Kisere South	KiS	**48**	-
Kisere Center	KiWW	**17**	-
Kisere Fogging	KiF	7	-
Kisere East	KiE	2	-
Kaimosi	Kai	**19**	-
sum		616	148

3.4. Molecular Analyses

3.4.1. DNA-Extraction

Extraction of genomic DNA was conducted by using a silica-gel-membrane technology, which allows a simple and fast isolation and provides intact and highly pure nucleic acids from a variety of sample sources. VOGELSTEIN & GILLESPIE (1979) firstly described the adsorption of nucleic acids to the surface of glass or silica in the presence of high concentrations of chaotropic salts. The procedure has been conspicuously improved and refined and complete Miniprep-Kits are available nowadays (e.g. QIAGEN®, MACHEREY NAGEL®). The principal procedure goes according to the following description.

After crushing the starting material in a microcentrifuge tube, samples are lysed with proteinase K. Buffer conditions are adjusted to provide optimal DNA-binding conditions. The lysate is then loaded onto the silica-gel membrane incorporated into a microspin column. During centrifugation the DNA is selectively bound to the membrane, while contaminants like polysaccharides and proteins do not bind and pass through. Remaining contaminants and enzyme inhibitors such as proteins and divalent cations are removed in two washing steps. The bound nucleic acid is washed with alcohol containing buffers for desalting. Pure DNA is then eluted in low salt buffer or water (GAUCH *et al*. 1998).

Genomic DNA was extracted from legs (*Amphitmetus transversus*) or the thorax (*Monolepta vincta*) of single adults, which were preserved in 100 % ethanol and stored at –18 °C. Extraction was performed using the DNEASY®TISSUE KIT (QIAGEN®) as well as the NUCLEOSPIN®TISSUE KIT (MACHEREY-NAGEL®).The isolation and purification was performed according to the manufacture's protocol (QIAGEN: "DNeasy Protocol for Animal Tissues"; MACHEREY-NAGEL: "Standard protocol for human and animal tissue"). In the end the results were checked on a 1.5 % agarose gel using ethidium bromide as a staining compound. The isolated genomic DNA was stored at –18 °C.

3.4.2. PCR Amplification

In order to achieve an adequate amount of DNA for further analyses an accumulation of the DNA sequences of interest is necessary. This can be done by the polymerase chain reaction (PCR), in which the thermostable Taq-polymerase allows the duplication of the

template against the number of the conducted cycles. (MULLIS & FALOONA 1987). The PCR, which was originally conceived as a tool in DNA diagnosis of genetic diseases has revolutionised the molecular facilities and has a broad field of applications, including the direct use in explorative analyses like sequencing, restriction cleavage or hybridization (AERT *et al.* 1998). It can also be used for the direct interpretation of size differences by gel electrophoresis as, for example, in analysis of length polymorphism of microsatellites. In the following PCR methods were used in the range of cloning, sequencing and microsatellite analyses.

The principle of PCR combines the enzymatic function of the Taq-polymerase in synthesising DNA-sequences with the mathematical concept of geometrical progression. There are three major steps during the process of the PCR: In the denaturation step the reaction volume is heated up to 94 °C. At this temperature the double stranded DNA melts open to single stranded DNA. During the annealing step the temperature is reduced to 45–60 °C. The primers attach to the denaturated template and the Taq-polymerase starts copying the template. During the extension step the reaction volume is kept on a temperature of 72 °C. This provides the ideal working temperature for the Taq-polymerase. The DNA strand is elongated by coupling the complementary bases (dNTPs) of the template to the primer on the 3' side. The three steps are repeated for 30–40 cycles automatically. Both strands are copied during the PCR and therefore an exponential increase of the number of DNA-copies against the number of the conducted cycles can be theoretically expected. Actually, the generation of a PCR product is more complex than straightforward exponential accumulation (AERT *et al.* 1998). While there are specific priming sites at the original DNA template specific termination sites are missing. Since this template remains in the solution, the amount of a specifically terminated product of distinct length increases linearly. That is an important point in analysing length polymorphisms, which depends on the detection of the accurate length of an allele. During the second cycle the initial priming sites will become termination points in the complementary strand. The first amplified complementary strand therefore forms the new template, which leads to the exact length of the subsequent PCR product (Fig. 8).

Factors influencing the PCR are not only the natural features of the DNA template (G+T content, concentration, length of region to be amplified), the properties of the DNA polymerase (stability, concentration), and the condition of the primers (size, composition, sequence) but also the concentration of the dNTPs, the ionic environment ($MgCl_2$, KCl) as well as organic compounds (formamide, glycerol, DMSO) and the temperature profile of the PCR (annealing time and temperature, extension time, number of cycles).

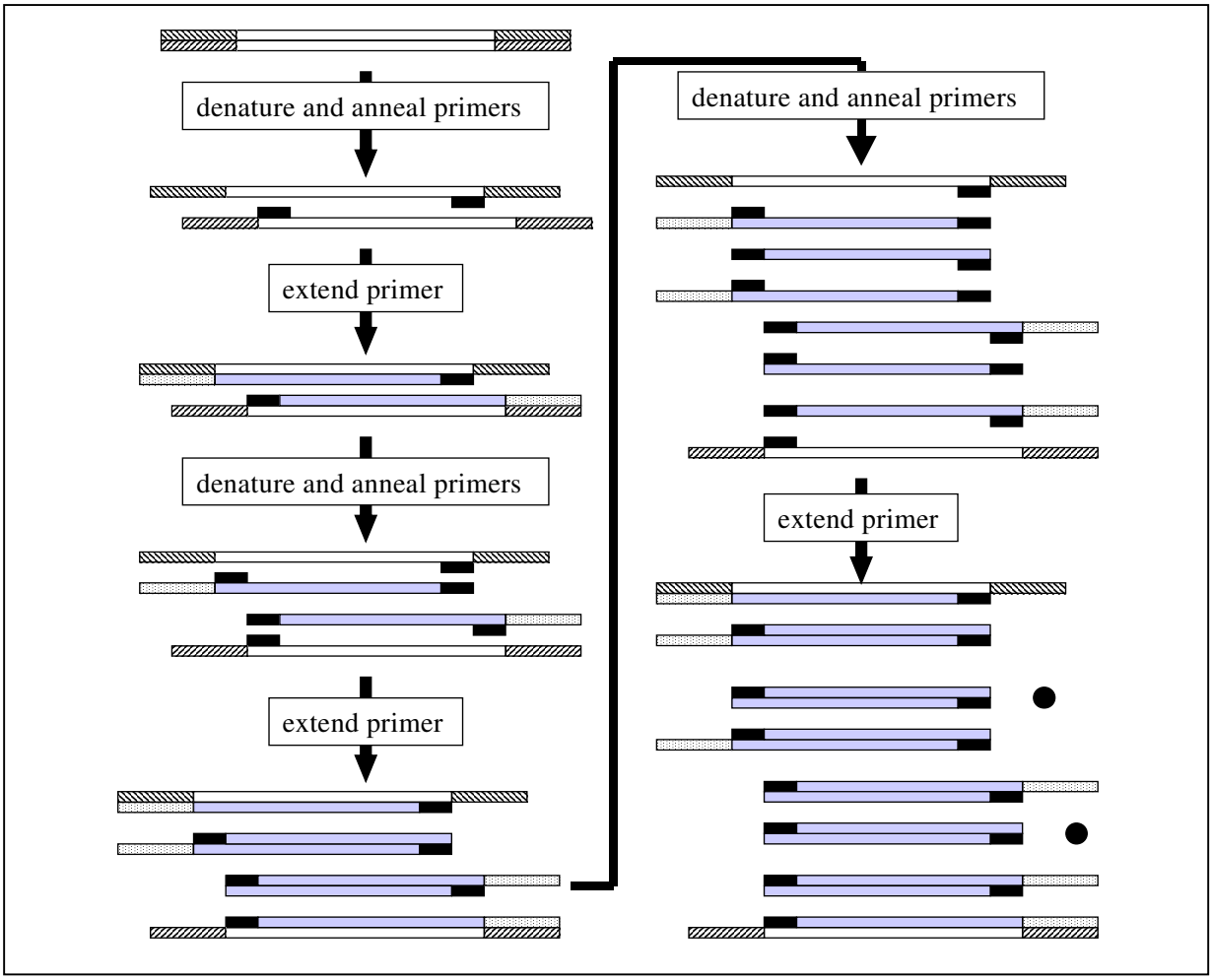

Fig. 8: Amplification process of PCR. The segment to be amplified in the template is uncoloured. The rest are shown as segments with slashes. Primers are shown as filled boxes. The new synthesized target region is marked blue, while additionally synthesized parts are shown as stippled bars. The first bona fide PCR product appears during the third cycle and is indicated with a black point. Scheme follows AERT *et al.* (1998).

The following protocols for PCR amplification were used for the respective procedure in this study (Table 3, 4; Fig. 9, 10). Tables indicate the concentration of component and the used volume for one PCR. Further details are given in the single protocols of the applied methods. PCRs were performed on a GENEAMP®2700 thermal cycler (APPLIED BIOSYSTEMS®), a GENEAMP®9600 thermal cycler (APPLIED BIOSYSTEMS®) as well as a T-GRADIENT®thermal cycler (BIOMETRA®).

Results were checked on a 1.5 % agarose gel using ethidium bromide as a staining compound. Subsequently PCR products were purified using purification kits from PROMEGA® or SIGMA® and checked with agarose gel electrophoresis again in the case of the microsatellite plasmids.

Table 3: Composition of PCR reaction of microsatellite plasmids of *A. transversus* and *M. vincta*

Ingredients	**Volume [µl]**
ddH_2O	33.1
1.5 x PCR-buffer (without $MgCl_2$; contains 100 mM Tris-HCl, 500 mM KCl, pH 8.3; SIGMA®)	5.0
$MgCl_2$ (25 mM; SIGMA®)	5.0
dNTPs (2 mM mix, containing 2.5 mM of each dNTP (SIGMA®)	4.0
forward primer (10 mM)	0.8
reverse primer (10 mM)	0.8
Taq-polymerase (0.2 unit contains 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween 20, 50 % glycerol; SIGMA®)	0.3
genomic DNA (10-20 ng)	1.0
Total	50.0

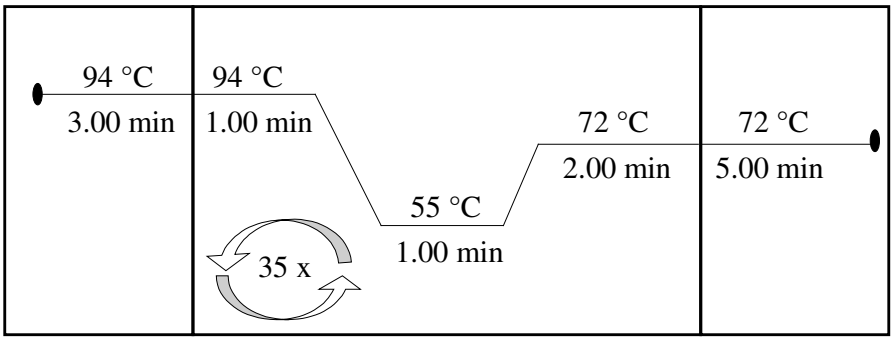

Fig. 9: Amplification scheme of applicated PCR for plasmid DNA.

Table 4: Composition of PCR reaction of microsatellite polymorphism analysis of *A. transversus* and *M. vincta*

Ingredients	**Volume [µl]**
ddH_2O	9.8
1.5 x PCR-buffer (without $MgCl_2$; contains 100 mM Tris-HCl, 500 mM KCl, pH 8.3; SIGMA®)	3
$MgCl_2$ (25 mM; SIGMA®)	2
dNTPs (10 mM mix, containing 2.5 mM of each dNTP (SIGMA®)	3
forward primer (10 mM)	0.5
reverse primer (10 mM)	0.5
Taq-polymerase (0.2 unit contains 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween 20, 50 % glycerol; SIGMA®)	0.2
genomic DNA (10-20 ng)	1
Total	20

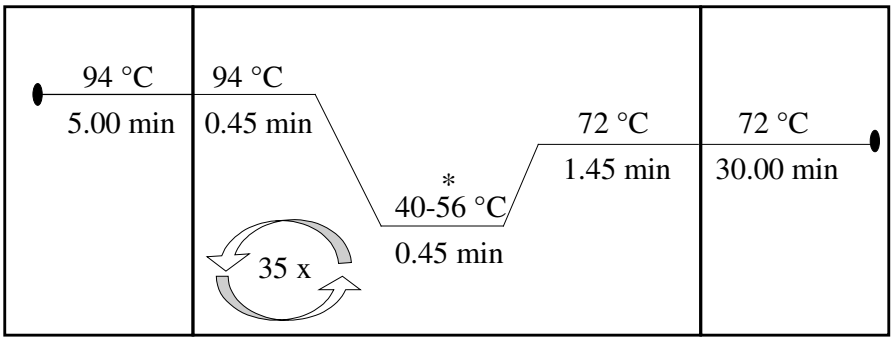

Fig. 10: Amplification profile of applied PCR for microsatellite analysis. * Respective annealing temperature depend on single marker according to Table 6 and 7

3.4.3. DNA Sequencing

3.4.3.1. Cycle Sequencing

The DNA sequencing requires a specific primer and a template, which are the starting points for the DNA-amplification. The main characteristics of the sequencing reaction developed by SANGER *et al.* (1977) is the continuously interruption of the amplification by the presence of 2'–3'didesoxynucleotides (ddNTPs) which can be randomly incorporated beside the natural desoxinucleotids (dNTPs). Similar to the PCR the cycle sequencing reaction consists of three steps. First the double stranded DNA is denaturated at 94 °C. During the annealing progress (50 °C) only one primer is used, which attaches to the single strand of the DNA. In the extension step (60 °C) the primers are elongated by the Taq-polymerase. Normally 72 °C is the optimal working temperature of the enzyme, but because it has to incorporate ddNTPs, which are chemically modified with a fluorescent label, the temperature is lowered (Fig. 11).

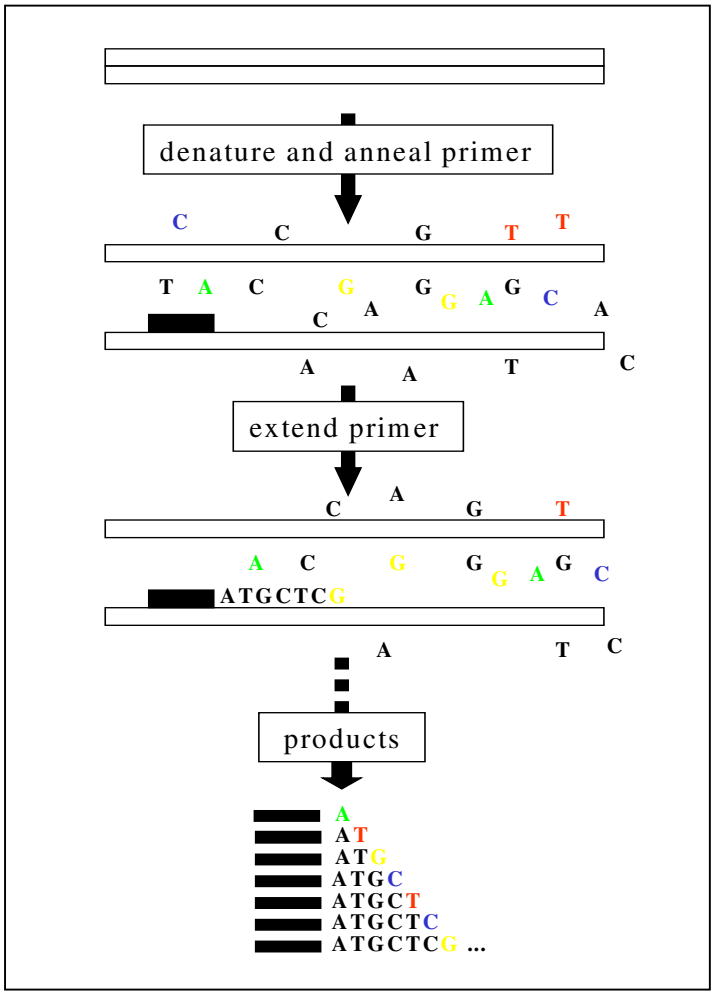

Fig. 11: Principle of cycle sequencing. Black letters indicate dNTPs, coloured letters indicate fluorescence labelled ddNTPs, which terminate elongation.

The complementary bases to the template are coupled to the 3' end of the primer adding either dNTPs or ddNTPs randomly. When a ddNTP is incorporated, the chain elongation is terminated selectively at A, C, G or T, because a ddNTP lacks a 3'-hydroxyl group. Since the ddNTPs are fluorescently labelled it is possible to detect the colour of the last base of this fragment on an automated sequencer (see below). Due to the presence of one primer, only one strand is copied during a sequencing cycle and a linear increase of the amplification product appears.

3.4.3.2. Gel electrophoresis

During the cycle sequencing reaction, a mixture of DNA strands with different length are produced and have to be separated in order to obtain the correct sequence of the respective DNA fragment. Fragments of different size can be separated by means of an acrylamide gel electrophoresis. DNA has a negative charge and migrates to the anode of the electric field. Smaller fragments migrate faster through the gel pores, so the DNA molecules are separated on their size. The fragments, which are fluorescent labelled by the last attached ddNTP are passing a laser beam at the bottom of the gel. Each nucleotide emits a characteristic wavelength (ddATP = green, ddTTP = yellow, ddCTP = red, ddGTP = blue) after excitation by an argon–laser. The light is collected and focussed by lenses into a spectrograph. Based on the wavelength, the spectrograph separates the light across a CCD camera (charge coupled device). Each base emits its characteristic colour and the sequencer can detect the order of the bases in the sequenced gene (Fig. 12).

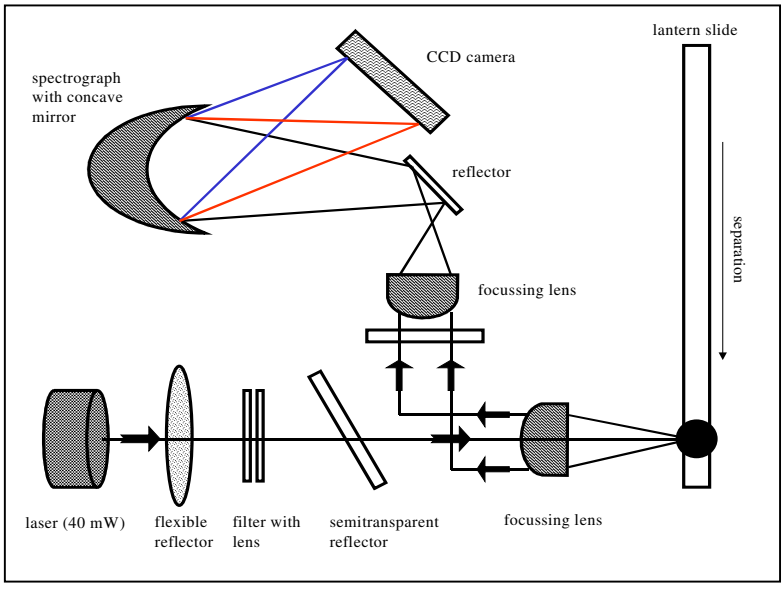

Fig. 12: Principle of detection on an ABI PRISM®377 sequencer. Scheme follows manual of APPLIED BIOSYSTEMS

The following cycle sequencing protocol was used for the sequencing of plasmid clones during the procedure of isolation of microsatellites in *Amphitmetus transversu*s and *Monolepta vincta* (Fig. 13). The table indicates the concentration of components and the used volume for one cycle sequencing reaction (Table 5). The reaction was performed on a GENEAMP®2700 thermal cycler (APPLIED BIOSYSTEMS®) as well as a T-GRADIENT®thermal cycler (BIOMETRA®).

Table 5: Cycle sequencing protocol of *A. transversus* and *M. vincta* for plasmid sequencing.

Ingredient	Volume [µl]
ddH_2O	3.5
ABIPRISM®BIGDYE™ Terminator Cycle Sequencing Kit (APPLIED BIOSYSTEMS)	3.0
$MgCl_2$ (25 mM; SIGMA®)	1.0
Universal M13 primer (10µM)	1.5
DNA (product from plasmid PCR)	1.0
Total	10.0

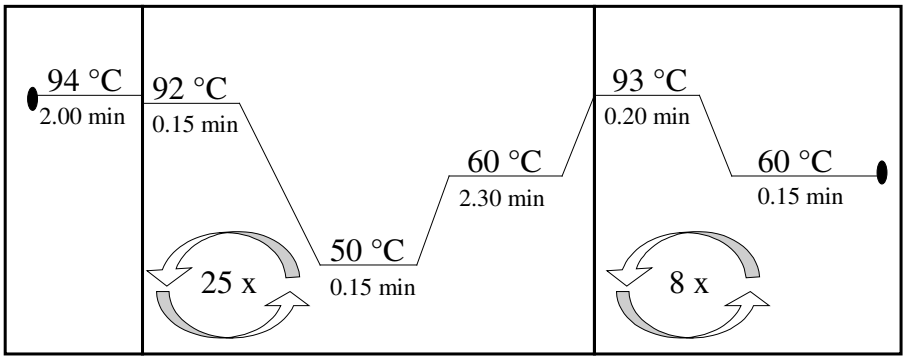

Fig. 13: Amplification profile of cycle sequencing reaction for microsatellite plasmids.

The cycle sequencing reaction products were cleaned and precipitated by using an ethanol protocol. The electrophoresis of the products was carried out on 5 % polyacrylamide gels using an ABI PRISM®377 sequencer. The procedure followed the manufacturer's protocol. The sequences were analysed using DNA SEQUENCING ANALYSIS SOFTWARE ™ VERSION 3.4.1. (ABI PRISM®) (Fig. 14).

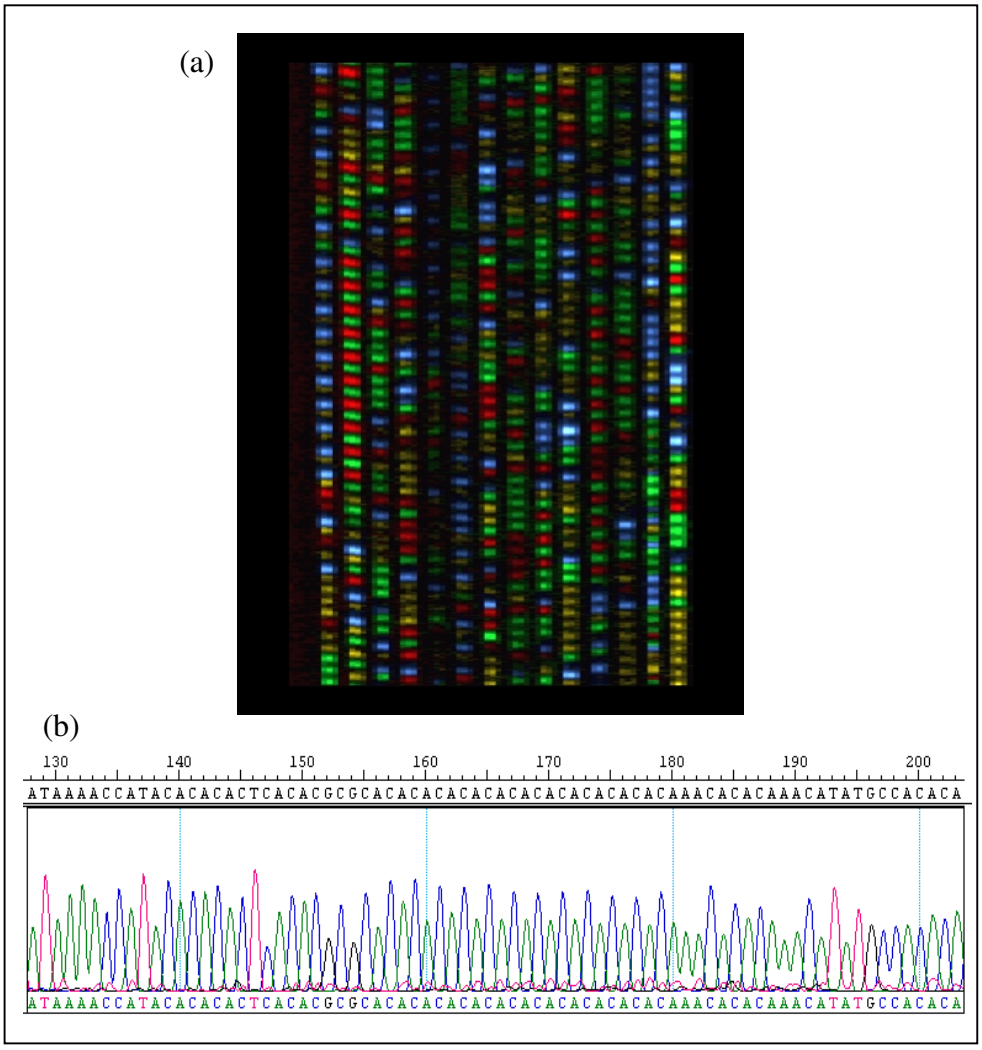

Fig. 14: Screenshot of a sequence run on a polyacrylamide gel (a). Electropherogram of a DNA sequence containing a microsatellite (b).

3.4.4. Isolation of Microsatellites

Microsatellites have to be isolated *de novo* from most species being examined for the first time. They are usually located in noncoding regions where the nucleotide substitution rate is higher than in coding regions. Therefore, the strategy of designing universal primers matching conserved sequences in general is not possible for microsatellites (ZANE *et al.* 2002). Consequently, cross amplifications for microsatellite sequences in nearly related taxa is limited. Analyses for birds have shown a 50 % success rate in cross-amplification and detection of polymorphism in species which diverged approximately from 10 to 20 Ma (PRIMMER *et al.* 1996, MOORE *et al.* 1991).

A widely used isolation method is based on selective hybridization. The basic protocol was presented by KARAGYOZOV *et al.* (1993). During the procedure genomic DNA is fragmented by restriction enzymes. The fragments are then ligated to a known sequence, a vector or an adaptor. Afterwards, the fragments are optional amplificated by PCR in order to yield a larger amount of DNA. Subsequently the sequences are hybridized to a repeat containing probe. Fragments containing repeat motives are bound to 5' biotinylated probes and captured with streptavidin coated magnetic beads (KIJAS *et al*. 1994). After the hybridization step and several washes to remove non specific binding, the DNA is eluted and increased by PCR amplification. Finally the enriched DNA is cloned into a suitable vector. Recombinant clones can be directly sequenced and analysed for the presence of repeat motives (Fig. 15). The basic protocol has been modified by various authors and single steps differ.

DNA of the present study was extracted using a DNEASY®TISSUE KIT (QIAGEN®) and checked qualitatively on a 1.5 % agarose gel using ethidium bromide as a staining compound. The enrichment of the microsatellite library was done by a commercial supplier (BIOPSYTEC ANALYTIC GMBH), who isolated microsatellites using a selective hybridization protocol and provided 100 plasmids and glycerine cultures for further analyses. During the isolation, the genomic DNA was restricted using the enzyme HinfI. Subsequently, adapters for PCR amplification were ligated. Biotin marked $(CA)_{10}$ probes were then hybridized to the fragments and such hybridized DNA-sequences were isolated by streptavidin beads. The selected fragments were amplified by PCR using the adapter's sequence as primer position and transformed into the PCR®2.1-TOPO®plasmid vector (INVITROGEN®). After a transformation of bacterial cells with the ligation product, 100 plasmids were isolated. The plasmid-inserted fragments were amplified using universal M13 primer according to the protocol given in chapter 3.4.2. in order to facilitate the subsequent sequencing. After purification the PCR products were used in the cycle sequencing reaction. The protocol is given in chapter 3.4.3.. There have been 65 plasmids sequenced of *Monolepta vincta* and 87 of *Amphitmetus transversus* on an ABIPRISM®377 DNA sequencer using the ABIPRISM®BIGDYE™ Terminator Cycle Sequencing Kit (APPLIED BIOSYSTEMS). Sequences were processed using the Software Package 'STADEN' (STADEN 1996). In *Monolepta vincta* about 32 (= 49 %) of the sequences contained repetitive motives from eight to 22 repeats. In *Amphitmetus transversus* about 43 (= 49%) of the sequences contained repetitive motives from eight to 87 repeats. In the following primers were designed using the program PRIMER

VERSION 0.5 (DALY *et al.* 1991). Sixteen forward and reverse primers were created for *Amphitmetus transversus* and thirty-three for *Monolepta vincta*.

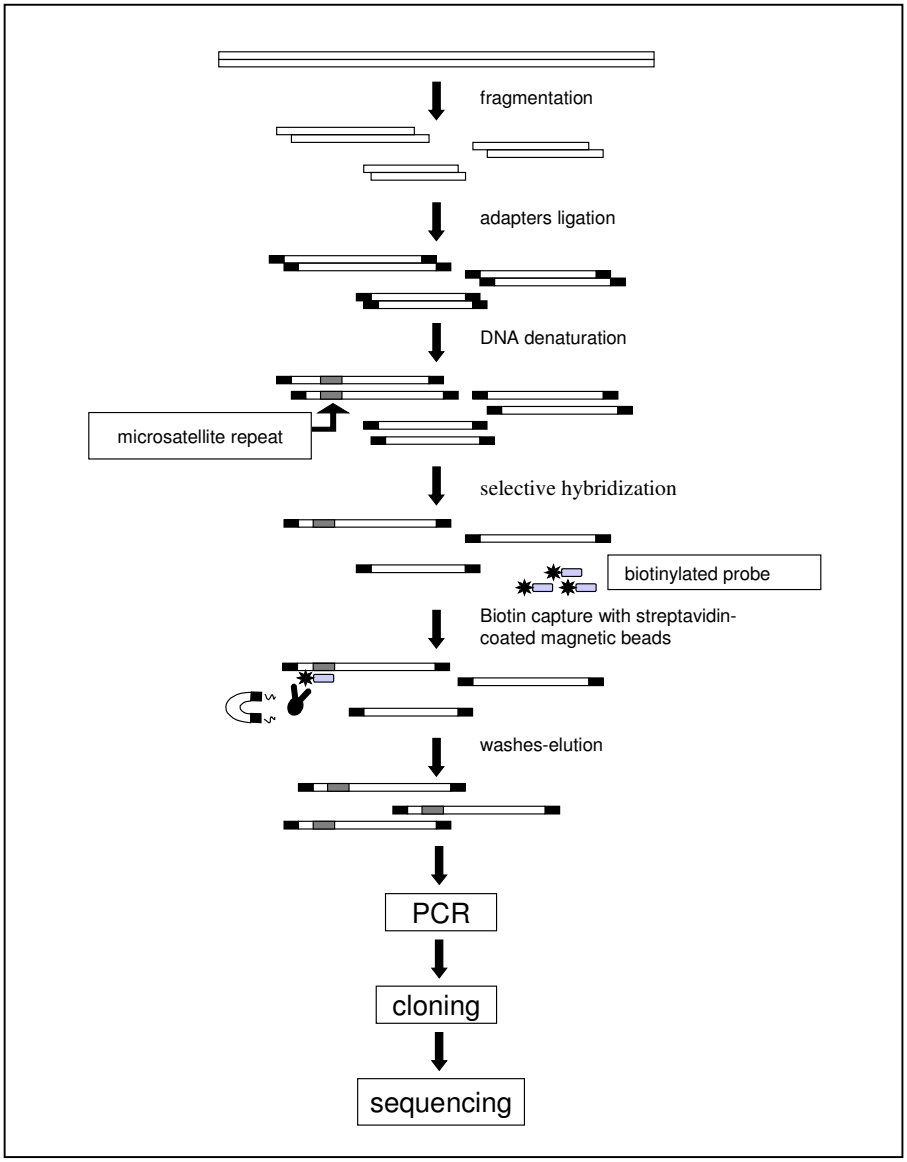

Fig. 15: Scheme of the hybridization protocols according to ZANE *et al*. (2002).

Each forward primer was labelled at the 5' end with either FAM, JOE or TAMRA by the supplier (SIGMA-ARK). Primer pairs were tested on a set of 100 randomly chosen individuals of nine different populations distributed over Kakamega Forest in case of *Amphitmetus transversus* (PATT *et al*. 2004) and on 140 individuals of seven different populations in case of *Monolepta vincta* (PATT *et al*. in press). Six tested loci have been detected as polymorphic in *Amphitmetus transversus* and nine in *Monolepta vincta*. They presented fragments of high quality and clearly distinct alleles. The primer sequence, repeat motive, the number of alleles at each polymorphic locus, their size range and the respective annealing temperature for PCR are shown in Table 6 and 7.

Table 6: Characterization of *Amphitmetus transversus* microsatellite markers. Ta = annealing temperature during PCR; F = forward; R = reverse.

Micro-satellite Locus	Repeat Motive	Predicted Product Length (bp)	Primer Sequences	T_a (°C)	Number of Alleles	Size Range (bp)
At-MS05	$(TG)_3(TG)_{11}$	145 bp	F: 5' CTACGACCCGCGTTCTGC	56 °C	5	137–151
			R: 5' TTATACTGATAATGACGTATG			
At-MS42	$(TG)_{15}$	343 bp	F: 5' TAATGTAACAATAAAGTCTGC	56 °C	3	340–344
			R: 5' AGCGCCTAGTGCCATTGTA			
At-MS58	$(CA)_9$	322bp	F: 5' AATGCATTTTTCTTACCA	50 °C	4	308–324
			R: 5' CTTATGATGCCGTTAGG			
At-MS90	$(CA)_{10}$	246 bp	F: 5' CCAAAGAGACAAGGAGAA	56 °C	3	246–256
			R: 5' GTGCGAACTACGGTATTATCAT			
At-MS91	$(TG)_{10}$	134 bp	F: 5' AATGCTGAGCCTTATCCA	56 °C	5	134–144
			R: 5' AACGTCTCTTTTCTTCTTATTC			
At-MS93	$(TG)_{11}$	337 bp	F: 5' CCTCCAACCGATCTTTCCTAC	56 °C	8	323–341
			R: 5' CTGGGCGACACTTTCTTACG			

Table 7: Characterization of *Monolepta vincta* microsatellite markers. Ta = annealing temperature during PCR; F = forward; R = reverse.

Micro-satellite Locus	Repeat Motive	Predicted Product Length (bp)		Primer Sequences	T_a (°C)	Number of Alleles	Size Range (bp)
Mv-MS04	$(TG)_8$	128 bp	F:	5'-GAACTTTCGTAAAAAAAGACTAC	56 °C	3	116–120
			R:	5'-CCGATTAACATTACTTCCCAG			
Mv-MS06	$(CA)_{17}$	135 bp	F:	5'-TACAGTATGTGGTAAATAGCG	56 °C	17	115–155
			R:	5'-CGGTCTTCTGCTGCTCATC			
Mv-MS11	$(TG)_{11}$	241 bp	F:	5'-AAGATTTTTAAGCGATGATA	56 °C	6	229–247
			R:	5'-AGGAGCTGCTAGTTTCTGAG			
Mv-MS15	$(AC)_9$	151 bp	F:	5'-AGAAACATAAACAGCTCAAAGGAA	40 °C	5	147–153
			R:	5'-CAAAATGGAAATATAAACAGCAGA			
Mv-MS21	$(CA)_{12}$	206 bp	F:	5'-TACTTCGATTTCGCTAACAACTCT	40 °C	9	194–208
			R:	5'-AAAGGCTCAAATCAAATCCAGGTG			
Mv-MS43	$(AC)_9$	211 bp	F:	5'-GCTTTTGTTTATGACTTTTAGGTA	42 °C	18	223–257
			R:	5'-AATCACGTTTTCTTCTTAGTTTTA			
Mv-MS60	$(TG)_{10}$	218 bp	F:	5'-AGTTGACCTCTCCGTTCTAA	40 °C	10	215–250
			R:	5'-CCACGAAGGGTTGTAAAG			
Mv-MS81	$(CA)_9$	220 bp	F:	5'-CTAATGGAGATGGCACCTGA	40 °C	20	197–271
			R:	5'-TCTAGACGGGAAACCAAAAT			
Mv-MS84	$(TG)_8$	260 bp	F:	5'-TTATTTCTGACTTTATCCCCACTA	40 °C	7	220–265
			R:	5'-TTAAAAGAACTTGAGGCGAAATG			

3.4.5. Analyses of Polymorphism

Using newly designed primer pairs (Table 6 and 7) polymorphic microsatellite sequences were amplified and each individuals was genotyped by fragments of characteristic length. The mixture of DNA strands of different length were separated using an acrylamide gel electrophoresis (Chapter 3.4.3.), which allows to separate molecules of one base pair difference. The fragments were labelled by a fluorescent dye (FAM = blue, JOE = green, TAMRA = yellow), which was attached to the 5' end of the respective forward primer. By

mixing a size standard to each probe that contains labelled fragments of defined size (ROX = red), the absolute length of the fragment could be examined.

In total 624 individuals of *Amphitmetus transversus* were characterized with six as well as 148 individuals of *Monolepta vincta* with nine microsatellite markers. PCR was carried out following the protocol given in chapter 3.4.2.. Afterwards the PCR products were mixed up with GENE SCAN-500-ROX-SIZE-STANDARD (APPLIED BIOSYSTEMS®) according to the protocol (Table 8).

Table 8: Preparation of PCR products for gel electrophoresis

Ingredients	Volume [µl]
Formamid	1.5
Gene Scan-Rox-500-Size-Standard	0.3
Buffer	0.7
PCR-Product	0.5

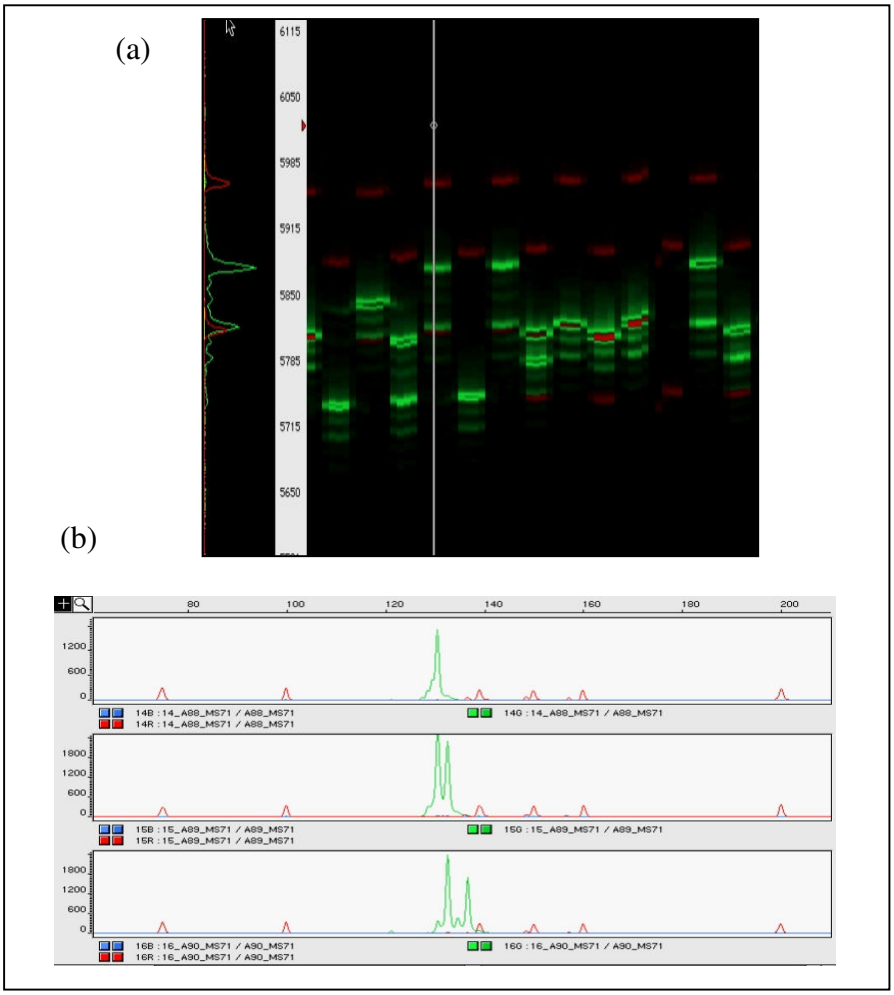

Fig. 16: Screenshot of a polyacrylamide gel electrophoresis of microsatellite fragments (a). Electropherogram of microsatellites in three different individuals (b). Green lines indicate microsatellite sequences of different length. Red lines represent size standard.

Probes were loaded on a 5 % polyacrylamide gel and separated by gel electrophoresis using an ABI PRISM®377 sequencer. Results were analysed with GENESCAN 3.1.2. (APPLIED BIOSYSTEMS®) according to the manufactor's protocol. Fragment length were scored using the program GENOTYPER®2.0 (APPLIED BIOSYSTEMS®) (Fig. 16). Fragment sizes, and hence genotypes were arranged in a matrix for further analyses (Appendix Table A1–A4).

3.5. Statistical Analyses

3.5.1. General Characterization of Markers and Populations

The advantageous properties of microsatellites are found in their dense distribution throughout eukaryotic genomes, their generally high level of polymorphism, and their relative ease of screening once isolated. Microsatellites constitute codominant markers, which are most powerful tools in analysing the genetic structure of a population because heterozygotes can be distinguished unequivocally from homozygotes. Currently, they are the most widely used genetic markers in evolutionary, ecological and conservation studies. Nevertheless, we also have to face difficulties in the interpretation of the information given by the marker system. An incorrect assignment of genotypes during the molecular analyses of microsatellites may cause a bias in population genetic results. Biases may concern estimates of allele frequency and population differentiation. Several reasons of an incorrect detection of genotypes are possible. Genotyping errors can be caused by problems with the quality or concentration of the DNA, slippage during PCR-amplification (SHINDE *et al.* 2003) or short allele dominance, because the amplification of alleles containing large repeat units fails (WATTIER *et al.* 1998). The most common problem in the analysis of microsatellites is the occurrence of null alleles (CALLEN *et al*. 1993, BEAUMONT & BRUFORD 2000). These alleles are not amplified due to a mutation at the primer site, which prevents primer binding during PCR. If null alleles are present, the observed proportion of homozygote genotypes is higher than the expected in an equilibrium population, because heterozygous individuals containing null alleles are screened as false homozygotes. Failure to comply with Hardy-Weinberg-Equilibrium can also be caused by biological reasons. Explanations include the possibility of population substructuring (WAHLUND 1928), assortative mating between individuals, natural selection on single genotypes or inbreeding effects. It is therefore necessary to analyse the

features of the marker and the composition of the populations cautiously in order to identify and discriminate those possibilities (BEEBEE & ROWE 2004).

Six microsatellites were developed for the apterous weevil *Amphitmetus transversus* and nine markers for the highly active leaf beetle *Monolepta vincta*. Sample sites that yielded an insufficient number of individuals or failed in the amplification of several markers were excluded from the consecutive analyses. The respective marker system was used to characterize 19 populations of *A. transversus* and six populations of the *M. vincta* distributed across the Kakamega Forest. Several tests were conducted to characterize the properties of the single loci as well as the composition of the populations.

3.5.1.1. Genetic variability of the microsatellite markers

The established marker systems of *A. transversus* and *M. vincta* as well as the genetic composition of the sampled populations were characterized by using basic parameters of genetic variability. In general, markers are defined as polymorphic when the most common allele has a frequency of less than 0.95 or 0.99 (HARTL & CLARK 1997). In this study markers were treated as polymorphic at a cut-off frequency of 0.99. The estimates of genetic variability were calculated for each sample site, as well as for each marker. Besides the observed and expected heterozygosity, the numbers of alleles are given. The parameters were calculated using the program GENEPOP 3.3 (RAYMOND & ROUSSET 1995).

3.5.1.2. Linkage Disequilibrium and Hardy-Weinberg Proportion

The basic properties of the considered markers were examined by testing the loci for linkage disequilibrium and for concordance with Hardy-Weinberg expectations. Linkage disequilibrium is a measure to determine the degree of association between two alleles. Assuming random mating, genotypes content alleles of any gene at random according to its frequency given by the Hardy-Weinberg proportions. If the alleles of the genes are associated by chance, the frequency of a gamete carrying any composition of alleles equals the product of those frequencies. Genes in random association are said to be in linkage equilibrium. If alleles at two distinctive loci occur in gametes more frequently than expected given the known allele frequencies, the alleles are stated to be in linkage disequilibrium (HARTL & CLARK 1997). Loci that are linked are assumed to be co-inherited due to their physical proximity on a chromosome. Linkage between markers is undesirable, showing that loci are not independent. Hence, fewer loci are available for analyses than expected.

The Hardy-Weinberg-Equilibrium (HWE) describes the proportion of a particular genotype expected in a panmictic population with known allele frequencies. The expected frequencies of homozygous and heterozygous genotypes on given allele frequencies are related by:

$$(p_A + q_a)^2 = p^2_{AA} + 2pq_{Aa} + q^2_{aa}$$

with p as the frequency of allele A and q as the frequency of allele a. If we assume the pooled individuals of a sample site to be representatives of a population or rather a deme, which is defined as a local interbreeding unit, we expect a balanced distribution of genotypes according to the Hardy-Weinberg expectations.

The genotype frequencies of *Amphitmetus transversus* and *Monolepta vincta* were tested for conformity to Hardy-Weinberg expectations for each locus and each sample site. Furthermore, the genotypes of all pairs of loci were tested for linkage disequilibrium. The analyses were performed using exact tests with significance determined by a Markov Chain method implemented in the program GENEPOP Version 3.3 (RAYMOND & ROUSSET 1995). Exact P-values are estimated according to GUO & THOMPSON (1992). All tests were conducted using 500 batches and 2000 iterations. An increasing number of batches and iterations reduces the standard deviation of the test. With each test, a sequential Bonferroni correction for multiple tests was applied (HOLM 1979, RICE 1989).

3.5.1.3. Test on Genotyping Errors and Re-Estimation of Allele Frequencies

Genotyping errors and null alleles normally show a specific pattern, which is reflected in the deficiencies of particular genotypes or loci and helps to discriminate those caused by genotyping errors and deviations due to non-panmixia.

Null alleles normally are segregated with common alleles at high frequency resulting in an excess of corresponding homozygotes. Rare alleles are not expected to show a homozygote excess, because the probability of segregating with a null allele depends on the allele frequency. The program MICRO-CHECKER (VAN OOSTERHOUT *et al.* 2004) calculates a cut-off frequency for which allelic classes are analysed to show a homozygote excess and are expected to segregate with a null allele. When most of those allelic classes show a homozygote excess, the program indicates the potential presence of a null allele. The deviations from Hardy-Weinberg-Equilibrium are compared across all loci in order to distinguish null alleles from real biological processes. It is interpreted as evidence for random mating and panmixia, if some loci are in Hardy-Weinberg-Equilibrium while other loci show

disequilibrium. In such cases, deviations from Hardy-Weinberg proportions are assumed to be locus-specific. Null alleles can bias the analyses of population structure as their identity and frequency may vary among populations (PEMBERTON *et al.* 1995). In general, there are three possibilities in handling the problem of null alleles. If enough loci are available markers containing null alleles are discarded or analyses of the specific marker are repeated using newly designed primers. If those methods are not feasible, frequencies of the null alleles can be estimated from the observed deficiency of heterozygotes for the given population, assuming that each population is in Hardy-Weinberg-Equilibrium. (NEWMAN & SQUIRE 2001).

Because evidence for the presence of null or non-amplifying alleles was found at several loci either in the marker system of *Amphitmetus transversus* as well as of *Monolepta vincta,* an estimate of the frequency of the null allele was calculated using the program MICRO-CHECKER (VAN OOSTERHOUT *et al.* 2004), while the frequencies of all other alleles were simultaneously re-estimated (CHAKRABORTY *et al.* 1992, BROOKFIELD 1996), assuming that the population is in Hardy-Weinberg-Equilibrium.

3.5.1.4. Allele Frequency Distribution

The distribution of allele frequencies was graphically illustrated for each locus of *Amphitmetus transversus* and *Monolepta vincta*. This offered the possibility of visualizing the proportion and distribution of the particular alleles according to the length of their repeat unit. The pattern may gives information concerning the underlying mutational process of the respective loci (LINDENMEYER & PEAKALL 2000). Furthermore, the proportion of alleles per population was displayed on a map of the Kakamega Forest in order to visualize allelic variation of single markers on the spatial scale.

3.5.2. Test on Genetic Diversity

Habitat loss and fragmentation reduces population sizes in the concerned areas and changes the spatial distribution of remaining subpopulations. Reduced population size may result in increased genetic drift and inbreeding, leading to a loss of genetic variability (WRIGHT 1931, NEI *et al*. 1975). Parameters which determine the magnitude of effects are influenced by the extent to which population size and migration rates are reduced as well as the time since isolation (SLATKIN 1987). Temporary but significant reductions in population

size are defined as bottlenecks. Although consequences of bottleneck effects on the reduction of genetic variation have been reported for several vertebrate and plant species in temperate and tropical climate (LEBERG 1991, COMPS *et al.* 2001, BELLINGER *et al*. 2003, RAMSTAD *et al*. 2004, SUMNER *et al*. 2004), there are only poor references for effects inferred from invertebrates in tropical raiforests. The dimension of random genetic drift in a population is inversely related to its effective size (N_e) (HARTL & CLARK 1997). Population densities of the majority of beetle species in tropical forest have been found to be considerable small (WAGNER 2000) and the effective population size (N_e) may even be substantially smaller than the census population size (FRANKHAM 1995). Short generation times of most invertebrates might even increase the rate at which reductions in populations sizes are reflected in the genetic data (CLARKE 2000).

The Kakamega Forest is a highly fragmented an partly degraded tropical rainforest and thus a well-suited area for such studies. The agricultural areas between fragmented sites of the forest are expected to constitute strong barriers to migration to the low mobile *Amphitmetus transversus*, which is only found inside the forest and is most likely unable to survive in open farmlands. The sampling of a number of populations of the weevil, which are located either in fragments as well as in the continuous forest of the Kakamega Forest, offer the possibility of analysing the effect of fragmentation events on the apterous invertebrate. Populations which are supposed to have undergone a decline by human induced fragmentation and degradation were compared with populations which are assumed to be demographically stable.

"Genetic erosion", the decrease in genetic variation of an isolated population due to random genetic drift and inbreeding, is supposed to appear within several genetic parameters. Gene diversity (NEI 1973) or expected heterozygosity, the mean number of alleles per locus and the percentage of polymorphic loci belong to the standard estimates of genetic diversity.

Additionally, some new approaches have been arisen within the last decade, concerning the alteration in the proportion of rare alleles (frequencies < 0.1) induced by bottleneck effects (ALLENDORF 1986). Resulting effects are reflected in an observed heterozygosity excess immediately after the decline (CORNUET & LUIKART 1996), a mode shift in allele frequency distributions (LUIKART *et al*. 1998) as well as decreasing M-ratios (GARZA & WILLIAMSON 2001). The occurrence of mode shifts of allele frequency distributions was tested (LUIKART *et al*. 1998), while observed heterozygosity excess (CORNUET & LUIKART 1996) as well as M-ratios (GARZA & WILLIAMSON 2001) were rejected, because the given dataset turned out not to be suitable.

Differences in allelic richness (Â), gene diversity (H_S), proportion of polymorphic loci (P) as well as mode-shifts were examined between populations in fragments (MalW, MalO, MalN, KiN, KiWW, KiS, Kai; N = 7) vs. populations located within continuous forest sites (Col, BusI, Iku, Buk, IsiI, Vih, YalI, Cam, Sal, IseI, BuyI, Kib; N = 12). With respect to the differences in size and quality of the four fragmented areas of Kakamega Forest, fragments were divided into two distinct categories. The three most distant fragments of sizes smaller than 200 ha and the largest fragment Kisere show different qualities. The former do not only differ from the latter in size and distance to the main forest, but also in their documented contemporary history. While Kaimosi and both fragments located at Malawa have undergone large anthropogenic induced changes by deforestation, logging and plantation, Kisere is the most unaffected part of the whole forest due to its sheltered location between two rivers. Therefore, the first category is composed of the populations located in the small fragments (< 200 ha, MalW, MalO, MalN, Kai; N = 4), whereas the second category consists of populations located in moderate fragment (> 400 ha, KiN, KiWW, KiS; N = 3). These two groups were then separately compared to populations located within the continuous forest site (Col, BusI, Iku, Buk, IsiI, Vih, YalI, Cam, Sal, IseI, BuyI, Kib N = 12). The significance level was set at $P < 0.05$, while probabilities of $P < 0.1$ were denoted as tendencies.

3.5.2.1. Allelic Richness

WRIGHT (1931) demonstrated that random genetic drift in small or rather bottlenecked populations has two major effects resulting in changes of allele frequencies as well as a loss of genetic variation. Among other things, these effects are reflected in the total number of alleles. This parameter is expected to be the most sensitive marker to recent reductions in populations size and hence to the status of variability (NEI *et al.* 1975, LEBERG 1992, 2002, SRIKWAN & WOODRUFF 2000, SPENCER *et al*. 2000).

The number of alleles, which is expected following a bottleneck (n'), is given by

$$E(n') = n - \sum_{i=1}^{n} (1 - p_i)^{2N}$$

(DENNISTON 1978) with n as the original number of alleles, p_i as the frequency of the i^{th} allele and N as the population size during a bottleneck. As resulting from the above equation, the loss of alleles largely depends on the effective size of the bottlenecked population.

In estimating this parameter one has to considere that the number of alleles per locus (A) depends on the number of sampled individuals (N) and therefore the interpretation of

interpopulation differences in the number of alleles can be complicated if based upon unequal numbers of samples (PETIT *et al*. 1998). The relationship between N and A is asymptotic, with the greatest effect of differences of N on A occurring when N is small. Additionally, the influence of N on A is bigger for loci with high polymorphism than for such with lower ones (LEBERG 2002). This is an important fact for highly polymorphic microsatellite loci. To circumvent this problem, a couple of solutions are available. One possibility is the assimilation of the data from different populations by the rarefaction method. Rarefaction can be used to compensate for differences in sample size and number, whereas the results are standardized to a constant number of individuals. The method was first developed for analyses of species diversity by HURLBERT (1971) and adapted to an estimation of allelic richness (Â) by El MOUSADIK & PETIT (1996). Allelic richness (Â) is denoted as the number of different alleles found when the specified sample size is collected at the locus in question. The principle is to estimate the expected number of alleles in a sub-sample of 2n genes, given that 2N genes have been sampled (N ≥ n). Allelic richness was calculated in the population genetic program FSTAT (GOUDET 2001), where n is fixed as the smallest number of individuals typed for a locus in a sample. Estimated Allelic Richness per locus and sample (Â) is then calculated as:

$$\hat{A} = \sum_{i=1}^{n} \left(1 - \frac{\binom{2N-N_i}{2n}}{\binom{2N}{2n}} \right)$$

where N_i is the number of alleles of type i among the 2N genes. Each term under the sum corresponds to the probability of sampling allele i at least once in a sample of size 2n. If allele i is so common that it is certain to sample it - when $2n > (2N-N_i)$ - the ratio is undefined but the probability of sampling the allele is set to 1 (GOUDET 2001). Estimates of Â obtained with rarefaction are similar to those obtained from multiple subsampling methods, and differences in precision are negligible (LEBERG 2002).

During the calculation of Â, the number of individuals per population was adjusted to 14 individuals. Differences among groups of populations for allelic richness and gene diversity were carried out using the "comparison among group"-option which is incorporated in FSTAT Version 2.9.3.2. FSTAT first calculates the average (χ) (over samples and loci) of the allelic richness for each group and then the difference between the chosen groups according to:

$$OS_{\chi} = \sum_{i=1}^{n\delta groups-1} \sum_{j=i+1}^{n\delta groups} (\chi_i - \chi_j)^2$$

The significance of the statistics OS_x, is assessed by performing a permutation scheme of 1000 iterations. Whole samples are allocated at random to the different groups (keeping the number of samples in each group constant), and S_x is calculated from the randomised data set. Subsequently, the P-value of the test is taken as the proportion of randomised data sets giving a larger S_x than the observed OS_x (GOUDET 2001).The examination of differences between multiple groups was additionally performed using an analysis of variance (ANOVA). Bonferroni was taken as Post-Hoc test to correct for multiple comparisons using the program SPSS 10.0.7. Kolmogorov-Smirnov tests were performed to test whether the data are parametric (ZAR 1999). In order to test for differences of the mean in comparison of single markers, t-test and a single factor ANOVA were used in case of parametric data as well as Mann-Whitney and Kruskal-Wallis in the case of non-parametric data (ZAR 1999). A Spearman Rank Test was conducted to determine if a relationship between fragment size and allelic richness exists.

3.5.2.2. Gene Diversity or Expected Heterozygosity

Gene diversity (H_S) (NEI 1973) is equivalent to expected heterozygosity for diploid data. It is defined as the probability that two randomly chosen alleles are different in a sample or rather that an individual is heterozygous at a given locus. Gene diversity is therefore calculated as

$$\hat{H} = \frac{n}{n-1}\left(1-\sum_{i=1}^{k} p^2_i\right)$$

where n is the size of the sample, k is the number of alleles, and p_i is the sample frequency of the allele A_i in the sample (NEI 1987). Thus, the greater the value of H is, the greater is the genetic variability of a population. In populations which are near mutation-drift equilibrium, the number and frequency of alleles at selectively neutral loci remain constant and hence also gene diversity. Mutation-drift equilibrium results from a dynamic equilibrium between mutation and genetic drift. It will be reached if the effective size of a population (N_e) remains stationary for 4–10 multiplied by N_e generations (NEI & LI 1976). Regarding the effect of random genetic drift on closed populations, the average heterozygosity and hence the variability changes following the dynamic described in the equation

$$H_t = H_o \left[1-\left(\frac{1}{2N_e}\right)\right]^t$$

with H_t as heterozygosity after t generations, N_e as effective populations size, H_0 as the original heterozygosity and t as the number of generations. H_t decreases at a geometric rate since H_t is multiplied by the constant $(1-{}^{1}/_{2Ne})$ each generation. In a population which is in state of mutation-drift equilibrium, new mutations cause a balancing increase of variability. A population which has undergone a decline in population size and therefore is not at equilibrium will experience a decrease of the average heterozygosity due to random genetic drift. The rate at which heterozygosity is lost after a population decline depends primarily on the new effective population size of the isolated population, while regeneration of variability through new mutations can usually be ignored (WRIGHT 1931).

It was tested whether populations of *A. transversus* in fragmented habitats indicate lower gene diversity than in habitats located in the continuous forest. The tests were conducted over all markers as well as for single markers. The parameters as well as the significance for differences in gene diversity between groups were computed using the program FSTAT Version 2.9.3.2 (GOUDET 2001). Testing for differences between pairs of groups, an analysis of variance (ANOVA) with a Bonferroni Post-Hoc test was performed. Analyses for differences regarding single markers were conducted by t-test and a single factor ANOVA.

3.5.2.3. Proportion of Polymorphic Loci

Beside the proportion of heterozygous individuals (H_S) and alleles (A) in a population, the proportion of polymorphic loci (P) counts to the most common measurements of genetic diversity (NEI *et al.* 1975, LEBERG 1992). In analysing allozyme data, LEBERG (1992) found that the proportion of polymorphic loci often reflects a population's history of bottlenecks.

The status of polymorphism gives important information concerning the evolutionary health of a population. Actually, the fixation on a single allele reflects an ultimate loss of genetic variability at the particular loci, which only can be compensated by new mutations or migration. High variable microsatellites often reveal a large number of alleles. A fixation on a single allele is therefore rarely found in that type of marker. In fact, the proportion of polymorphic loci at microsatellites turned out not to be sensitive to experimental bottlenecks in a study of SPENCER *et al.* (2000). However, the microsatellite loci of the given data set show an untypical low variability. The proportion of polymorphic loci (P) is therefore a promising measurement for the detection of recent bottlenecked populations in the case of *Amphitmetus transversus*.

It was tested whether populations in fragmented habitats reveal a mean higher proportion of fixed loci than populations located in the continuous forest. The tests were carried out for all markers. Differences between two groups were tested using an unpaired t-test, while comparisons among multiple groups were tested using an analysis of variance (ANOVA) with a Bonferroni Post-Hoc test. A Kolmogorov-Smirnov test was used to test whether data are parametric.

3.5.2.4. Mode-Shift – Changes in the Allele Frequency Distribution

Stochastic effects cause a faster loss of alleles at low frequencies compared to frequent ones at neutral loci (ALLENDORF 1986). Because drift acts harder on populations with small effective population size (N_e), the loss of rare alleles will be significant for populations which have been recently reduced in size compared to populations that are near mutation-drift equilibrium (NEI *et al.* 1976, CHAKRABORTY *et al*. 1980). A graphical assessment of whether a deficit in rare alleles exists in a sample of loci has been proposed by LUIKART *et al*. (1998). They showed that population bottlenecks cause a characteristic mode-shift distortion in the distribution of allele frequencies at selectively neutral loci. The method involves comparing the distribution of allele frequencies observed in a population suspected to have been bottlenecked, to the distribution expected in a population which is in mutation-drift equilibrium. For the graphical assessment, the alleles from polymorphic loci of samples are grouped into each of ten allele frequency classes and then plotted in a frequency histogram. The ten allele frequency classes are classified in decimals from 0.001 to 1. Following LUIKART *et al*. (1998), the low frequency class consisting of rare alleles is defined for allele proportions ranging between 0.001–0.1 and a high frequency class between 0.901–1 respectively. The other eight classes are denoted as intermediate frequency classes. A bottleneck is indicated if fewer alleles are found in the low frequency class than in one or more intermediate frequency classes. The bottleneck-induced distortion of the distribution of allele frequencies is characterized as a mode-shift.

According to LUIKART *et al.* (1998), bottlenecks are likely to be detectable for 40 to 80 generations, assuming the maximum bottleneck size to be detectable approximately 2 N_e to 4 N_e generations until genetic drift and new mutations begin to re-establish mutation-drift equilibrium (CORNUET & LUIKART 1996, NEI & LI 1976). Sample sizes of 20 to 30 individuals are unlikely to pretend that a population has been recently bottlenecked, while smaller samples are likely to miss alleles at low frequency (SJOGREN & WYONI 1994). The concerned population should not be substructured and had recent immigration, but should

show random mating (CORNUET & LUIKART 1996). Beside this, neutrality of the concerned loci must be given, because otherwise heterozygote advantage or balancing selection could maintain alleles at intermediate frequencies and thereby reduce the proportion of alleles at low frequencies. Although LUIKART *et al.* (1998) found no differences in testing on bottlenecks by including loci which show a deviation from Hardy-Weinberg assumption these marker should only be used with caution. As the given marker set is supposed to contain microsatellite loci with null alleles I tested for a modeshift pattern both with the given dataset and in consideration of calculated null allele frequencies in a second data set (Appendix, Table A1 & A2).

Mode shifts of allele frequencies among all loci within populations were calculated using the program BOTTLENECK (PIRY *et al.* 1999) and illustrated graphically for a qualitative analysis (LUIKART *et al*. 1998).

3.5.3. Test on Genetic Differentiation

As the migratory potential of individuals is limited in natural populations, dispersal preferentially occurs between geographically close populations, while genetic differences are inversely related to the amount of gene flow. The extent of this 'isolation by distance' pattern (1946, MALÉCOT 1948) depends in particular on the mobility and the dispersal potential of individuals as well as on the considered size and structure of the area. Populations of long-range dispersive species are supposed to be less differentiated in continuous habitats, because continuous gene flow among the distributional range prevents differentiation through random genetic drift. Additionally, the genetic differentiation among the populations of a species is largely influenced by the physical structure of the population range. The presence of particular barriers limits gene flow between adjacent populations. Molecular tools provide an effective mean of quantifying the effects of landscape structure on the geographical patterns of genetic variation. Populations separated by physical barriers are found to be more genetically dissimilar than populations in continuous habitats (SOKAL & ODEN 1978, MANNI *et al*. 2004). These facts allow predicting the effects of anthropogenic impact on the genetic population structure of a species. It is expected that isolation of habitat patches results in an increased genetic differentiation among populations (WRIGHT 1931). The development of the concept of barriers has largely influenced the investigation of the genetic population structure in relation to the spatial structure. The effect of barriers to gene flow has been discussed repeatedly in the literature concerning human populations (BARBUJANI & SOKAL 1990, SOKAL

& ODEN 1988). It is expected that barriers overlap with zones of rapid genetic change and several approaches have been developed for the detection of boundaries preventing gene flow. The goal is to locate areas where the rate of change of gene frequencies is particularly high (DUPANLOUP *et al.* 2002). The influence of geographical separation on the genetic population structure of populations has been documented for many plant and animal species (e. g.: POUNDS & JACKSON 1981, ARTER 1990, KEYGHOBADI *et al.* 1999, CULLEY & GRUBB 2003), including several studies on beetles (KING 1987, DESENDER *et al.* 1998, BROUAT *et al.* 2003, BRITTEN *et al.* 2003, KELLER *et al.* 2004). Studies concerning effects of landscape structure on beetle populations were carried out in temperate zones, while the genetic structure of beetles in tropical rainforests has not been documented so far.

The Kakamega Forest is structured in many respects. The aspect of the forest is characterized by apparent geographical barrier of anthropogenic induced fragmentation, which separates parts of the forest by agricultural landscape. Natural barriers like major river systems dissect the forest in unconnected parts. Beside of physical barriers also ecological differences within the range of the forest can be mentioned as reflected in the composition of the vegetation as well as the soil structure. SEWALL WRIGHT'S F-statistics was calculated following WEIR & COCKERHAM (1984) and used to measure genetic differentiation within and among sample sites of populations of *Amphitmetus transversus* and *Monolepta vincta*. On the basis of the concept of 'isolation by distance' it was tested whether geographical distance contributes to genetic distance (HUTCHINSON & TEMPLETON 1999). The extensive sampling of the weevil *Amphitmetus transversus* enabled detailed analysis of the spatial population structure. It was possible to test various hypotheses about the operating process to bring about the observed variation in allele frequencies. The fragmentation of parts of the forest as well as the natural structure of the environment may contribute to genetic differentiation by preventing gene flow in some directions. In the following, the applied methods in testing genetic differentiation between populations as well as the examination of causes are explained in detail.

3.5.3.1. Test on Genetic Differentiation

When a population is divided into isolated subpopulations, the heterozygosity in subpopulations declines compared to undivided populations. The decline in the number of heterozygote individuals due to subdivision within a population has usually been quantified using WRIGHT'S F-statistics (WRIGHT 1921). The F-statistics allow to partition heterozygote deficiency into a "within" and an "among" population component and equals the reduction in

heterozygosity expected with random mating at any one level of the population hierarchy relative to another (HARTL & CLARKE 1997). Wright's F-statistics quantify the departure from Hardy-Weinberg-Equilibrium at three levels. F_{IS} measures the heterozygote deficit within populations, F_{ST} among populations, and F_{IT} the global deficit of heterozygotes (GOUDET 2001). F_{ST} is therefore the most inclusive level of the population hierarchy and can be defined as:

$$F_{ST} = \frac{(H_t - H_s)}{H_t}$$

where H_t is the heterozygosity of the total population and H_s is the average heterozygosity over all subpopulations. Similar equations are valid for each level of the hierarchy. The statistics are based upon the infinite allele mutation model (IAM) (KIMURA & CROW 1964), which assumes that each mutation results in a new allele. Identical alleles share the same ancestry and are identical by descent (IBD). During the process of mutation, information about the ancestral state of the allele is erased and it is assumed that alleles rather not share a sequential history.

However, microsatellites (STRPs) are short tandem repeat polymorphisms and mutation results in the addition or deletion of repeat units. The process is reflected in a length polymorphism (LITT & LUTY 1989, TAUTZ 1989) that is supposed to be generated by a slippage mechanism during DNA replication (LEVINSON & GUTMAN 1987). The size of a new allele always depends on the size of the original allele. Hence, microsatellites are supposed to follow a stepwise mutation model (SMM) (KIMURA & OHTA 1978, JARNE & LAGODA 1996). Under this scenario, each mutation creates a novel allele either by adding or deleting a single repeat-unit. It follows that alleles of largely different size will be more distantly related than alleles of similar size. Markers obeying the stepwise mutation model are characterized by high levels of size homoplasy (ESTOUP *et al.* 1995). An estimator of genetic differentiation among populations which is based on a stepwise mutation model is R_{ST} (SLATKIN 1995), which accounts for the variance in allele size.

Although SMM seems to be a more realistic model to the mutation process of microsatellite evolution the estimation of R_{ST} has its drawbacks and is expected to give more accurate differentiation estimates than F_{ST} only under a strict SMM, a high number of markers as well as an increasing number of populations (GAGGIOTTI *et al.* 1999, BALLOUX & LUGON-MOULIN 2002). However, if deviations from the SMM occur and sample sizes are small the variance of the estimate is rather high and R_{ST} reflects population differentiation less accurate than F_{ST} (BALLOUX & LUGON-MOULIN 2002, ESTOUP *et al.* 2002). F_{ST} has been found to be

the most appropriate estimate when the number of loci analysed is less than 20 and the number of samples is less than 50 (GAGGIOTTI *et al.* 1999), which is the case for all sampled populations of *A. transversus* as well as *M. vincta*. Furthermore, the pattern of detected allele sizes in *A. transversus* and *M. vincta* bring about some doubt concerning the underlying mutation process. Each marker shows differences between at least two alleles that reflect an addition or deletion of more than one repeat unit. The loci At-MS93 does not show the expected allele distribution at all, as a 30 bp insertion/deletion between different states of alleles is obvious (Appendix Fig. A1 and A2). Under these requirements it was decided to omit the calculation of population differentiation on the R-statistics, because the F-Statistics, seems to be more appropriate.

The F-statistics were calculated according to WEIR & COCKERHAM (1984) using the program FSTAT (GOUDET 2001). These statistics are similar to the F-statistics of WRIGHT (1951), except that the method of WEIR & COCKERHAM incorporates the effect of small and uneven sample sizes (WEIR & COCKERHAM 1984). The parameters are related by

$$f = \frac{(F- \theta)}{(1- \theta)}$$

and correspond to the parameters of Wright's F-statistics with $F = F_{IT}$, $\theta = F_{ST}$ and $f = F_{IS}$ (WEIR & COCKERHAM 1984). The significance of the test was estimated by jack-knifing over samples and loci and bootstrapping over loci (GOUDET 2001). Additionally, exact tests were performed for testing significance of genetic differentiation between pairs of populations using FISHER'S (1954) method. Significance levels were Bonferroni corrected to account for multiple testing and to reduce Type I errors.

3.5.3.2. Test on 'Isolation by Distance'

The model of 'isolation by distance' (WRIGHT 1943, 1946, MALÉCOT 1948, 1950) describes the accumulation of local genetic differences under geographically restricted dispersal and shows that measures of genetic differentiation at neutral loci will increase with geographically distance in an equilibrium population. 'Isolation by distance' (IBD) is expected in populations with ongoing gene flow and the specific pattern can be used to distinguish those populations from isolated populations due to historical separation (SLATKIN 1993). In a group of populations at equilibrium it is expected that the average $F_{ST,}$ measured between pairs of populations, increases as a function of distance between populations.

Furthermore, variance in F_{ST} among site pairs also increases and thus the importance of drift relative to migration (HUTCHINSON & TEMPLETON 1999, BEEBEE & ROWE 2004).

The statistical analyses of the relationship between geographical and genetic distances is done by Mantel statistics, which offer a possibility of comparing two or more sets of distance measures. The simple Mantel test considers two matrices, whereas the association between these matrices is computed by a permutation approach (MANTEL 1967). If the first matrix (A) contains information about the genetic distance among combinations of n populations and the second matrix (B) contains measures of physical distance in the same manner with *i* as the respective index of the row and *j* the index of the column, then the Mantel statistics (M) are computed as the sum of the products of the elements A_{ij} and B_{ij}, except for the diagonal elements $i = j$:

$$M = \sum_{i \neq j} A_{ij} B_{ij}$$

If the distances in matrix *A* are independent from the distances, for the same object, in matrix *B* the constituted null hypothesis is confirmed. The null hypothesis is tested by a Monte Carlo randomization in which the original value of the statistics is compared to the distribution found by permutating the rows and corresponding columns randomly in one of the matrices whereby the other matrix is hold tight. The correlation between the matrices is measured as the classical Pearson correlation coefficient ("r"), which tests a linear relationship between the matrices:

$$r = \frac{1}{N-1} \sum_{i=1}^{N} \sum_{j=1}^{N} \left(\frac{(A_{ij} - \bar{A})}{s_A} \right) \left(\frac{(B_{ij} - \bar{B})}{s_B} \right)$$

N is the number of elements in the matrix, $\bar{A}$ is the mean of *A* elements and S_A is the standard deviation of *A* elements. At the beginning of the procedure the reference value rAB is calculated. By permutating the rows and the corresponding columns of one matrix randomly, a new matrix A' is created and the correlation coefficient rA'B is computed. The randomization process is repeated a great number of times, whereas the generated coefficients constitute the reference distribution under the null hypothesis. The precision of the result is determined by the number of the repeats (≈ 1000 for $\alpha = 0.05$; ≈ 5000 for $\alpha = 0.01$; ≈ 10000 for greater precision (MANLY 1997)).

Two matrices, one containing estimates of the genetic distance and the other containing information of the geographical distance were generated and a pattern of IBD was examined. The correlation was tested using mantel statistics in the program ISOLDE, which

is implemented in the program GENEPOP (RAYMOND & ROUSSET 1995). The significance was checked by conducting 1000 permutations.

3.5.3.3. Examining Causes of Genetic Differentiation

The Kakamega Forest is structured in many respects and therefore the association of the genetic distance matrix, based on the extent of genetic differentiation between populations of *A. transversus,* to several matrices containing information about forest structure, was analysed. Simple and partial Mantel tests were used to measure the influence of landscape features on geographical pattern of genetic variation (SMOUSE *et al.* 1986, MANLY 1986). The correlation between a matrix of pairwise F_{ST} and several matrices containing information about landscape structure was examined. It has been shown that geographical distances are significantly correlated to genetic distances and causes an 'isolation by distance pattern'. This result was taken into consideration in testing other hypothesis concerning the pattern of differentiation along particular barriers. Each hypothesis has been analysed in a simple as well as in a partial Mantel test to account for effects of geographical distances. On the other hand, also the pattern of genetic distance against geographical distance might be biased by other factors. Hence, a partial Mantel test was also conducted for geographical distance regarding the particular hypothesis.

In comparison to the simple Mantel test the partial Mantel test considers three different distance matrices. Essentially, the partial Mantel test allows a comparison to be made among two variables while controlling the third. If a matrix of genetic distances is tested against a matrix of environmental distances, the spatial distance can not be neglected. Close sample sites tend to have similar environments, so that environmental and spatial distances will often be positively related. Furthermore, the geographical distance is expected to be correlated to the genetic distance ("isolation by distance"). A positive association between environmental and genetic distances may be consequently caused by spatial effects. Hence, using the procedure of a partial Mantel test, it is possible to remove those spurious correlations. The procedure is based on the use of multiple regressions (SMOUSE *et al.* 1986, MANLY 1986). The precision of the result is determined by the number of the matrix permutations, which is recommended to be more than 5000 repeats in case of the partial Mantel test (BONNET & VAN DE PEER 2002).

In this study, each matrix of environmental distance represents a different hypothesis regarding the particular route through the landscape along which gene flow might occur

(KING 1987, ARTER 1990), whereas the landscape variables are expressed in a nominal system of at least two characters.

Hypothesis I – Fragmentation: The Kakamega Forest is characterised by a high extent of fragmentation, and some parts in the periphery of the forest are separated by agricultural landscape. It was tested whether non-forested areas act as barriers to gene flow between the populations of *A. transversus* causing those samples separated by them to be more genetically distant than non-fragmented samples. Therefore, a matrix with a value of zero to pairs of populations which are not fragmented, as well as a value of one to pairs of populations which are separated by agricultural area was created (Appendix Table A9).

Hypothesis II - Riverine barriers: It is expected that major rivers act as barriers to gene flow and prevent gene flow between separated sites. Two main rivers pass through the Kakamega Forest from the eastern to the western side. The Isiukhu River is located in the northern, while the Yala River is located in the southern part of the forest. The rivers separate forested areas completely and are therefore supposed to act as barriers to gene flow on the apterous weevil. It was tested whether pairs of populations separated by the rivers are genetically more distant than non-separated. A matrix with a value of zero to pairs of populations which are not separated by a river, a value of one to pairs of populations which are separated by one major river as well as a value of two to pairs of populations which are separated by two major rivers was constructed (Appendix Table A6).

Hypothesis III - Ecological differentiation: The Kakamega Forest is ecologically structured, which is, among other things, reflected in soil structure as well as the composition of the vegetation. The ecological differences run from the northern part to the southern part of the forest, although the areas of differentiation in vegetation and soil structure are not congruent (Fig. 17, 18). If the causes of the ecological differentiation also play a role in the population genetic differentiation of *Amphitmetus transversus,* it is expected that individuals inhabiting the same ecological area mate more likely and/or have more mating success than those of different areas. Hence, pairs of population located at areas of different vegetation types as well as soil structure are expected to be more genetically dissimilar than pairs of populations located at similar ecological sites. The forest shows a changing composition of the vegetation depending on the particular succession stage of the regarding area, but also areas belonging to the same succession stage in the northern and the southern main part of the forest show a varying vegetation pattern. To test whether these ecological differences cause a significant genetic differentiation in populations of *A. transversus* a matrix for populations located in areas of the same succession stage was created. The sample sites in the fragments

Malawa and Kaimosi were not taken into account, because they were incommensurable due to the different succession stages and the greater impact of human activities. The analyses on the differences in the soil structure are based on a Reconnaissance Soil Map (Fig. 18). It was discriminated between pairs of populations located at sites of identical soil structure and those of different soil structure. A matrix was created with a value of zero to pairs of populations which are located in the same vegetation (soil) area and a value of one to pairs of populations which are located in different vegetation (soil) areas (Appendix Table A7 and A8). The significance of the test was achieved by permutating the matrices 10 000 times. The tests were conducted using the program Mantel3 (GOUDET 1991).

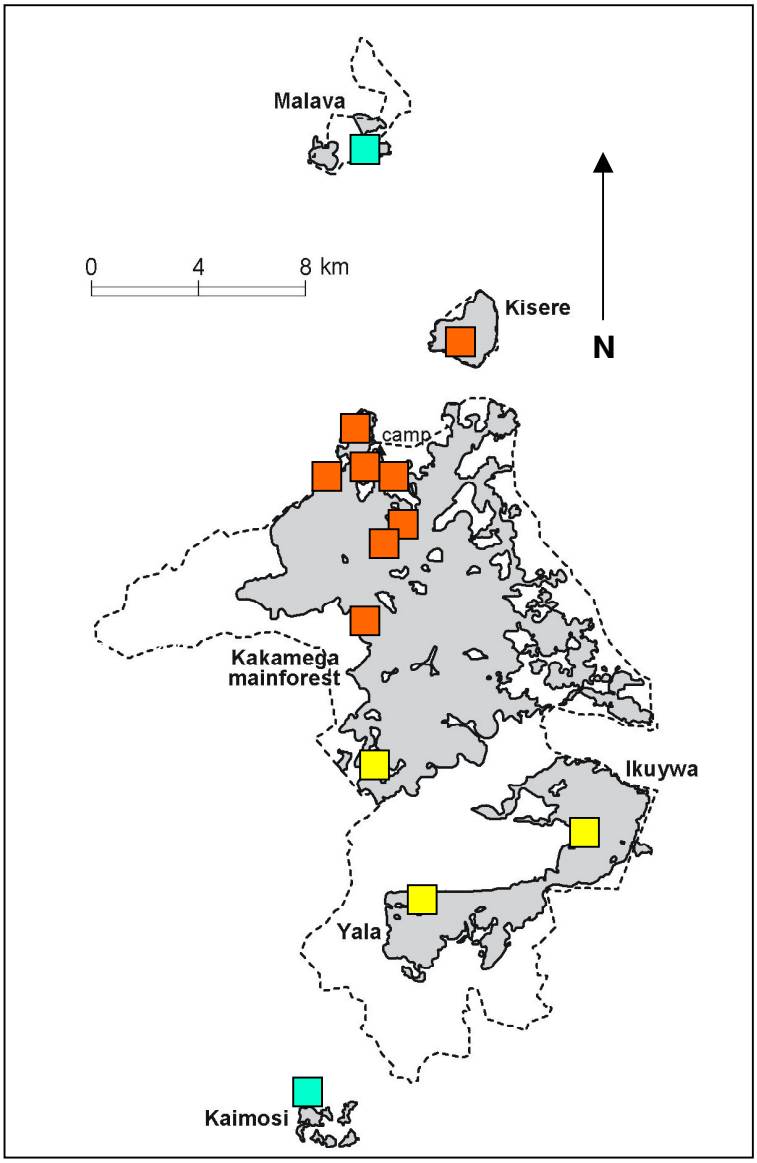

Fig. 17: Succession stages and composition of the vegetation in the Kakamega Forest. Data from ALTHOF, BIOTA East Project 04. Red squares = secondary forest; dominant species: Antiaris toxicaria – Funtumia africana; Yellow squares = secondary forest; dominant species: *Craibia brownii – Croton megalocarpus – Celtis mildbraedii;* blue squares = heavily logged and planted forest.

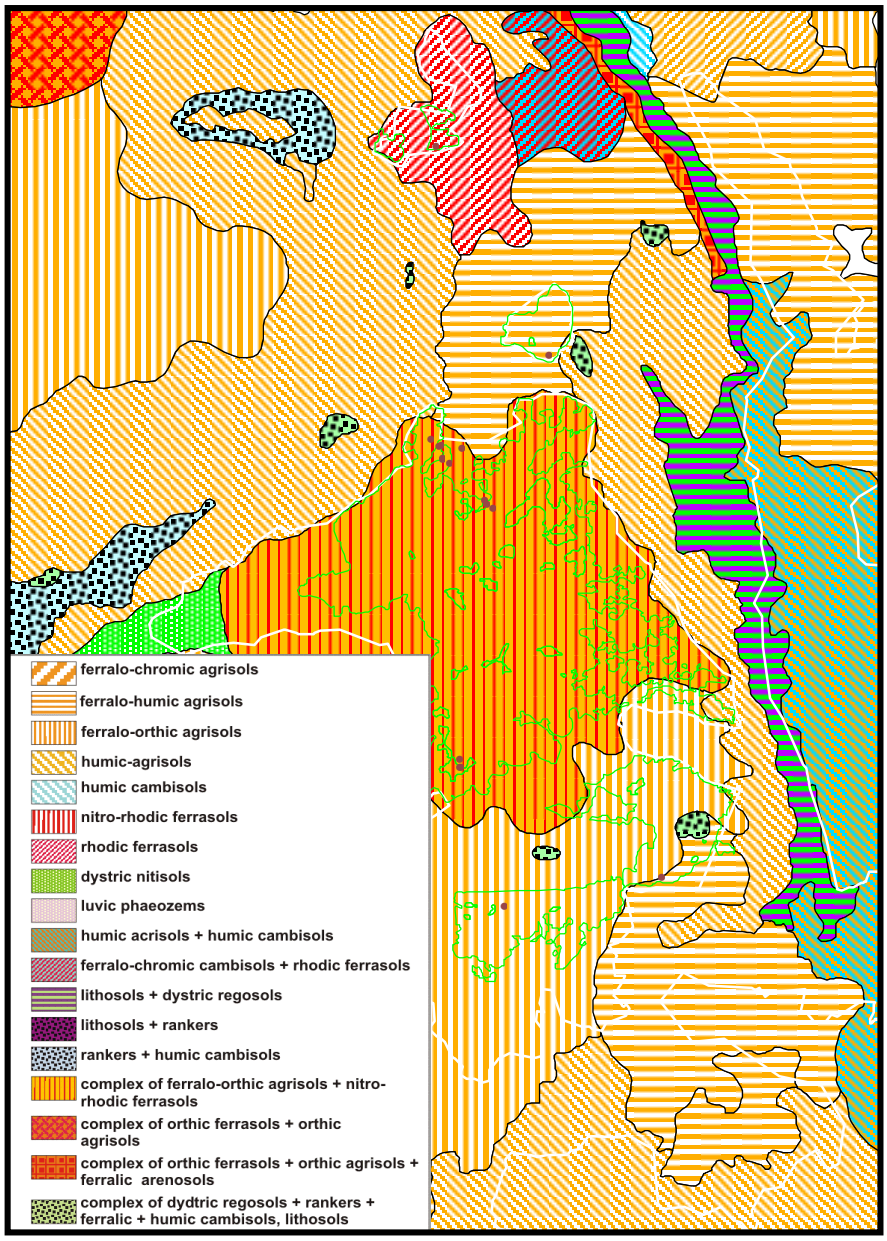

Fig. 18: Reconnaissance Soil Map of the Lake Basin Development Authority Area, Western Kenya 1:250 000 (1985). green outline = forest boundaries, white outline = official border of the forest. Source: Biota East-Africa – SCHAAB (E02).

3.5.3.4. The Detection of Genetic Barriers

While the principle of the Mantel statistics is based on the test of a particular hypothesis concerning the spatial structuring of the landscape that can be falsificated or verificated, other approaches offer the possibility of analysing the geographical arrangement of the genetic variability of a species by identifying geographic barriers to gene flow. Two methods, which are based on different approaches in identifying barriers to gene flow, were executed during this study.

The first method is based on the Monmonier's maximum difference algorithm (MONMONIER 1973), which was implemented in the program 'BARRIERS' (MANNI *et al.* 2004). The algorithm detects edges which are associated with highest rate of change in a given distance measure (e.g. F_{ST}) and is applied to a genetic network connecting all sample locations by a DELAUNAY triangulation (BRASSEL & REIF 1979). The DELAUNEY triangulation is the most direct way to connect adjacent points on a map and can be derived from the VORONOI diagram (VORONOI 1908). These diagrams imply that all possible points inside a polygon are closest to its centroid (which represents the sample point) than to any other. By this procedure, the geographic space S is divided in m subspaces S_i satisfying the following properties

$$\bigcup_i S_i = S$$

$$S_i \cap S_j = \varnothing \qquad \forall\ i \neq j$$

$$\text{DIST}\ (x_k, w_i) < \text{DIST}\ (x_k; x_j) \qquad \forall\ i \neq j,\ x_k \varepsilon\ S_i$$

with w_i as centroid of S_i. As a result, a network connecting all sample points is obtained. The boundary, more precisely, the area where the change of a distant variable is largest, is traced vertical to the edges of the network. It starts from the edge for which the distance value is maximally and proceeds across adjacent edges until it reaches the end of the map or completes a whole circle. The statistical significance of the computed barriers is obtained by a resampling procedure based on multiple matrices. A score is associated to the barriers constituting edges indicating how many times each one is included in one of boundaries computed from the N matrices. The score is indicated by the thickness of the given boundary.

The barriers were computed using matrices of F_{ST} values. To assess the statistical significance, a set of 50 matrices was resampled by a jack-knife analysis (MANNI & GUÉRARD 2004). Fifty matrices were created by selecting a proportion of 64-70 % of the raw data matrix each. The individuals were randomly chosen using BEN (MISOF, unpublished). Genetic distances between pairs of populations were calculated on the new created raw matrices. The program searched for the two strongest barriers to gene flow. The number of barriers seemed to be most adequate, because by computing more barriers on a set of fifty matrices the representation of the barriers is going to be chaotic and the interpretation of the bootstrap scores becomes quite difficult. On the other hand one might miss an important barrier to gene flow by regarding only the strongest border.

A major drawback of the method is that only adjacent populations are examined. Borders are marked where two contiguous sample sites are genetic dissimilar. This is especially a problem for the given sample design, where borders are relatively short due to the

oblongness form of the Kakamega Forest and hence, the arrangement of the sample sites within. This leads to direct association of particular populations to only one or two other populations and barriers are supported by genetic distances between considerable small numbers of sample sites. It was decided to apply a second method that relays on a different approach, in order to compare the results to those obtained by the Monmonier's maximum difference algorithm.

The applied method consists of a simulated annealing approach implemented in the program SAMOVA (DUPANLOUP *et al.* 2002), which defines groups of populations that are maximally differentiated from each other by maximizing the proportion of total genetic variance due to differences between groups. Genetic barriers are revealed as a by-product. Populations are assigned into groups with the constraint to be geographically adjacent and genetically homogenous. Similarly to the procedure of BARRIERS, sample localities are assembled into a DELAUNEY Network (BRASSEL & REIF 1979). Genetic distances (pairwise F_{ST}) are then calculated between all pairs of sample sites that are directly connected. At the beginning of the spatial analysis of molecular variance (SAMOVA), a random partition of n sampled populations is divided into K different groups. By maximizing the F_{CT} index in a simulated annealing procedure, the 'real' composition of the K groups is investigated by the proportion of total genetic variance due to differences between groups of populations (EXCOFFIER *et al.* 1992). The simulated annealing algorithm (KIRKPATRICK *et al.* 1983) uses a random search that not only accepts changes in decrease (or increase) of a function in the optimization process, but also changes that lead to suboptimal results. Hence, the procedure avoids becoming trapped at a local optimum. As time goes by, the departures from the detected optimum decreases underlying the assumption that one gets closer to the global optimum the more steps have been already performed.

At first an edge at random is selected on a given barrier between the specified groups. The two adjacent populations are identified and one population is chosen by random and assigned to the other group. The genetic barrier is modified and the F_{CT}^* value associated with the new partition is computed. The new structure is accepted with the probability

$$P = \begin{cases} 1 & \text{if } F_{CT}^* \geq F_{CT} \\ e^{(F_{CT}^* - F_{CT})S^A} & \text{if } F_{CT}^* < F_{CT} \end{cases}$$

with S as the number of steps performed and A as an arbitrary constant (A = 0.9158) (DUPANLOUP *et al.* 2002). This operation is repeated 10 000 times. To ensure that the final configuration of the K groups is not affected by the initial formation, the simulated annealing

process is iterated 100 times, starting each time from a different subdivision of the samples. The association with the largest F_{CT} value resulting after the 100 simulated annealing processes is retained as the best partitioning of populations (DUPANLOUP *et al.* 2002). The F_{CT} value represents the differentiation among the finally defined groups by the SAMOVA algorithm, while the F_{SC} value shows the extent of differentiation within these groups. The F_{ST} value indicates genetic differentiation among all populations. The significance level for both analyses was determined by repeating the simulated annealing approach 1000 times.

3.5.3.5. Population Phenogram on NEI's Genetic Distance

The parameter of genetic distance gives the extent to which populations differ from one another with respect to allele frequencies or DNA sequences at particular loci. Relationships between populations based on the genetic distance between each pair of a population can be obtained by NEI'S standard genetic distance (D_s) parameter, which is based on the infinite allele model. If p_i and q_i are the frequencies of the i^{th} allele in populations X and Y, respectively, and x_i and y_i be the corresponding sample frequencies, then NEI'S (1972) genetic distance is defined as

$$D = - \ln \left(\frac{G_{XY}}{\sqrt{G_X G_Y}} \right)$$

with G_X, G_Y and G_{XY} as means of Σp^2_i, Σq^2_i and $\Sigma p_i q_i$ over all loci, respectively. Usually, D is calculated by replacing population's gene identities G_X, G_Y and G_{XY}, by sample gene identities, J_X, J_Y and J_{XY}, which are the averages of Σx^2_i, Σy^2_i and $\Sigma x_i y_i$ over the r loci studied. An unbiased estimate of D is obtained by using the unbiased estimates of G_X and G_Y (NEI 1978), where $\hat{G}_X$ and $\hat{G}_Y$ are the averages of $(2n_x J_x - 1)/(2n_x - 1)$ and $(2n_y J_y - 1)/(2n_y - 1)$ over the studied loci.

Trees can be constructed by clustering procedures from matrices of genetic distances in several ways. One possibility is the **u**nweighted **p**air-**g**roup **m**ethod with **a**rithmetic mean (UPGMA). This method assumes that the considered sequences evolve at the same rate. A matrix of all pairwise genetic distances is used to build a tree. In a first step, the two populations with the smallest distance are grouped together. Afterwards, a new matrix is built, with the clustered species now considered as one unit. In the following analysis the new distance matrix is again searched for the smallest distance, and the grouping again occurs. This procedure is repeated until all populations are clustered into a tree. Tree building

methods do not only produce a tree topology, but also give estimates of branch length of the tree (HARTL & CLARK 1997).

The relationship of the populations of *A. transversus* calculated on NEI'S (1978) genetic distance was computed with the program POPGENE 3.2. (YEH & BOYLE 1997). On this information, a dendrogram was constructed and displayed in the program TREEVIEW 1.6.6. (PAGE 1996).

3.6. Software

The following software was used during the statistical analyses:

- BARRIERS Version 2.2 (MANNI *et al.* 2004)
- BEN (MISOF, unpublished)
- BOTTLENECK (CORNUET & LUIKART 1996)
- Excel for Windows 2000 (Microsoft Corporation)
- FSTAT Version 2.9.3.2 (GOUDET 1995)
- Genepop (RAYMOND & ROUSSET 1995)
- MANTEL3 (GOUDET 1991)
- MICRO-CHECKER Version 2.2.1 (VAN OOSTERHOUT *et al.* 2004)
- SAMOVA Version 1.0 (DUPANLOUP *et al.* 2002).
- SPSS for Windows (SPSS Inc., 2000, version 10.0.7)
- TREEVIEW Version 1.6.6. (PAGE 1996).

If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium. If insects were to vanish, the environment would collaps into chaos.

E. O. Wilson

4. Results

4.1. Population Genetics of Amphitmetus transversus

4.1.1. Genetic Variability of the Microsatellite Markers

Of the six microsatellite markers containing dinucleotid repeat motives that were developed for *A. transversus,* allelic diversity was calculated of 19 sample sites (Table 11). The total number of alleles per locus and population range from 1 to 8. The mean number of alleles per marker across all populations ranges from 1.58 (At-MS90) to 5.95 (At-MS93), while the mean number of alleles per population across all loci ranges between 2.00 (Kai) and 4.00 (KiS). The mean expected heterozygosity per locus and population ranges from 0.00 to 0.80 (At-MS93; Col), while the mean observed heterozygosity ranges from 0.00 to 0.79 (At-MS93; Sal). The highest variability in markers is found for At-MS93, while At-MS90 shows the lowest diversity. The most common allele of At-MS90 shows a frequency of 0.98 across all populations and a monomorphic occurrence in 11 of the 19 populations (Fig 22). Two other markers (At-MS05, At-MS91) are monomorphic in at least two populations. Across all loci 42 different alleles were detected with a maximum of 24 alleles at the population KiS.

4.1.2. Genotypic Linkage Disequilibrium and Hardy-Weinberg Proportion

In a test on the independence of the six loci no significant genotypic linkage disequilibrium was detected between any pair of loci across all populations as well as between any pair of loci for each population within 285 pairwise comparisons even without a sequential Bonferroni correction (Table 9). Therefore loci can be treated as independent in the following analyses. There is strong evidence for deviation from Hardy-Weinberg proportions at 3 of 6 loci (At-MS05, At-MS58, At-MS93; Table 10) as well as in 18 of 19 populations (Table 12) across all loci after sequential Bonferroni correction. Deviations were all heterozygote deficiencies. If heterozygote deficits are caused by inbreeding or WAHLUND-

Effects a deficit would be expected over all loci (MORAND *et al*. 2002). The partly occurrence of deficits at three loci might be caused by the presence of null alleles.

Table 9: Genotypic linkage disequilibrium for each locus pair across all populations in *Amphitmetus transversus.* NS = not significant.

Locus pair			Chi^2	d.f.	Significance
MS05	&	MS42	19.79	30	NS
MS05	&	MS58	22.52	30	NS
MS42	&	MS58	29.20	38	NS
MS05	&	MS90	6.46	12	NS
MS42	&	MS90	10.13	16	NS
MS58	&	MS90	5.59	16	NS
MS05	&	MS91	36.79	26	NS
MS42	&	MS91	36.64	34	NS
MS58	&	MS91	23.32	34	NS
MS90	&	MS91	4.61	16	NS
MS05	&	MS93	29.30	32	NS
MS42	&	MS93	31.09	38	NS
MS58	&	MS93	24.98	38	NS
MS90	&	MS93	13.82	16	NS
MS91	&	MS93	39.75	34	NS

Table 10: Hardy Weinberg exact tests for each locus across all populations in *Amphitmetus transversus.*

Locus	Chi^2	d.f.	P-value
At-MS05	186.70	28	0.000***
At-MS42	38.50	38	0.448
At-MS58	511.90	38	0.000***
At-MS90	17.80	12	0.124
At-MS91	38.10	28	0.097
At-MS93	64.50	38	0.005**

*** significance at P<0.001, ** significance at P<0.01

Table 11: Allelic diversity in *Amphitmetus transversus* populations with N = number of sampled individuals, n = number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity. Site code follows Table 2.

		At-MS05			At-MS42			At-MS58			At-MS90			At-MS91			At-MS93		Across all loci		
Site	N	n	H_O	H_E	n	H_O	H_E	n	H_O	H_E	n	H_O	H_E	n	H_O	H_E	n	H_O	H_E	n_{mean}	H_O	H_E
Col	50	2	0.00	0.04	4	0.44	0.47	3	0.06	0.39	2	0.04	0.04	4	0.21	0.23	5	0.63	0.72	3.50	0.23	0.31
BusI	29	2	0.03	0.03	4	0.53	0.58	2	0.00	0.28	2	0.03	0.03	2	0.40	0.33	6	0.70	0.72	3.00	0.28	0.33
Iku	23	3	0.13	0.40	3	0.32	0.52	2	0.00	0.50	1	0.00	0.00	3	0.17	0.43	8	0.70	0.77	3.33	0.28	0.44
Buk	28	3	0.07	0.26	5	0.65	0.26	2	0.00	0.34	2	0.04	0.04	2	0.04	0.04	5	0.08	0.67	3.17	0.20	0.27
IsiI	33	3	0.06	0.12	3	0.29	0.46	2	0.00	0.43	1	0.00	0.00	2	0.18	0.22	8	0.42	0.77	3.17	0.19	0.33
Vih	14	2	0.00	0.48	3	0.48	0.54	2	0.00	0.42	1	0.00	0.00	2	0.14	0.14	5	0.57	0.70	2.50	0.24	0.38
YalI	34	3	0.06	0.30	4	0.59	0.56	2	0.03	0.51	1	0.00	0.00	4	0.30	0.38	7	0.71	0.67	3.50	0.28	0.40
Cam	44	2	0.02	0.02	3	0.56	0.40	2	0.00	0.41	3	0.09	0.13	2	0.25	0.22	7	0.66	0.80	3.17	0.24	0.33
Sal	19	1	0.00	0.00	3	0.33	0.33	2	0.00	0.19	1	0.00	0.00	2	0.05	0.05	6	0.79	0.77	2.50	0.18	0.22
IseI	22	4	0.09	0.59	3	0.33	0.49	2	0.00	0.49	3	0.14	0.21	2	0.09	0.08	7	0.68	0.76	3.50	0.27	0.44
BuyII	25	1	0.00	0.00	3	0.6	0.29	2	0.04	0.25	1	0.00	0.00	2	0.13	0.25	7	0.71	0.70	2.67	0.20	0.25
Kib	23	4	0.30	0.61	3	0.78	0.46	3	0.04	0.52	1	0.00	0.00	2	0.35	0.29	7	0.61	0.71	3.33	0.29	0.43
MalN	19	1	0.00	0.00	3	0.26	0.52	2	0.00	0.34	2	0.00	0.10	2	0.21	0.19	5	0.68	0.73	2.50	0.23	0.31
MalW	24	2	0.04	0.25	4	0.11	0.36	2	0.00	0.38	1	0.00	0.00	1	0.00	0.00	4	0.71	0.58	2.33	0.17	0.26
MalO	24	3	0.08	0.16	3	0.64	0.16	2	0.04	0.36	1	0.00	0.00	1	0.00	0.00	4	0.67	0.65	2.33	0.16	0.22
KiN	22	2	0.09	0.24	2	0.33	0.33	2	0.00	0.51	1	0.00	0.00	4	0.36	0.35	4	0.64	0.65	2.50	0.25	0.35
KiWW	17	2	0.12	0.21	3	0.43	0.51	2	0.00	0.30	2	0.24	0.30	3	0.24	0.27	7	0.65	0.76	3.17	0.30	0.39
KiS	48	2	0.06	0.27	4	0.29	0.53	2	0.00	0.29	3	0.15	0.17	5	0.35	0.45	8	0.69	0.78	4.00	0.30	0.41
Kai	19	2	0.05	0.31	2	0.17	0.27	2	0.00	0.10	1	0.00	0.00	2	0.05	0.05	3	0.53	0.64	2.00	0.16	0.23
average		2.32	0.06	0.23	3.26	0.43	0.42	2.11	0.01	0.37	1.58	0.04	0.05	2.47	0.19	0.21	5.95	0.62	0.71			

Table 12: Hardy-Weinberg exact tests for each population across all loci in *Amphitmetus transversus.* The significance is Bonferroni adjusted.

Site	Chi²	d.f.	significance
Col	49.80	12	***
BusI	37.90	8	***
Kai	20.80	8	*
Iku	62.70	10	***
Buk	44.30	8	***
IsiI	71.70	10	***
KiN	41.80	10	***
KiWW	25.50	12	*
KiS	76.80	12	***
Vih	43.80	10	***
YalI	68.40	10	***
Cam	62.60	10	***
Sal	15.40	6	NS
IseI	70.20	12	***
BuyI	20.60	8	*
Kib	50.70	10	***
MalN	28.00	10	*
MalW	41.50	8	***
MalO	24.80	8	*

*** significant at P<0.001, ** significant at P<0.01, * significant at P<0.05, NS no significance

Three loci showed evidence for null alleles in the form of null homozygotes. These are individuals for whom no detectable PCR products could be observed at that particular locus, even with repeated attempts. The template from the same individual yielded simultaneously products at the most other loci. One null-homozygote was observed at locus At-MS05 for individual 15 in population 1 (Col) and at locus At-MS93 for individual 21 in population 12 (Cam). Four null-homozygotes were observed at locus At-MS58 for individual 25 in population 1 (Col), for individual 5 and 6 in population 9 (KiS) and for individual 21 in population 12 (Cam) (Appendix Table A1). Two individuals of population 1 (Col) did not yield amplification products at four markers, respectively. As this pattern is evident for several markers, the quality of the DNA seems to be the reason for the failed amplification. Although deviation from Hardy-Weinberg expectation is not significant at At-MS91 this marker tends to a heterozygous deficit, too.

4.1.3. Evidence of Non-Amplifying Alleles

In order to verify the assumption that non-amplifying alleles are responsible for the deviation of Hardy-Weinberg-Equilibrium all markers were tested for the presence of null alleles using the program MICRO-CHECKER (VAN OOSTERHOUT *et al.* 2004). Evidence for null-alleles was found in several populations at 4 of 6 loci (Table 13).

Table 13: Evidence for null alleles estimated with the program MICRO-CHECKER for populations of *Amphitmetus transversus*. Ho_E = total number of expected homozygotes, Ho_O = total number of observed homozygotes, NA = null alleles.

	At-MS05			At-MS42			At-MS58			At-MS90			At-MS91			At-MS93		
Site	NA	Ho_E	Ho_O	NA	Ho_E	Ho_O	NA	Ho_E	Ho_O	NA	Ho_E	Ho_O	NA	Ho_E	Ho_O	NA	Ho_E	Ho_O
Col	*	47.0	49.0		25.9	27.0	*	31.2	45.0		47.0	47.0		37.3	38.0		14.0	18.0
BusI		29.0	29.0		12.9	14.0	*	21.7	30.0		29.0	29.0		20.4	18.0		8.7	9.0
Kai	*	13.3	18.0		13.9	13.0		17.1	19.0		19.0	19.0		18.0	18.0		7.2	9.0
Iku	*	14.1	20.0		11.4	8.0	*	11.7	23.0		23.0	23.0	*	13.4	19.0		5.6	7.0
Buk	*	20.9	26.0		20.8	20.0	*	18.6	28.0		27.0	27.0		27.0	27.0		9.7	6.0
IsiI		29.2	31.0		18.1	17.0	*	19.1	33.0		33.0	33.0		26.0	27.0	*	7.9	19.0
KiN		16.8	20.0		14.8	13.0	*	11.0	22.0		22.0	22.0		14.4	14.0		7.9	8.0
KiWW		13.5	15.0		8.6	7.0	*	12.1	17.0		12.1	13.0		12.6	13.0		4.4	6.0
KiS	*	35.3	45.0		23.0	21.0	*	32.8	46.0		39.7	41.0		26.7	31.0		10.8	15.0
Vih	*	7.6	14.0		6.8	4.0	*	8.3	14.0		14.0	14.0		12.1	12.0		4.5	6.0
YalI	*	23.8	32.0		15.4	14.0	*	17.0	33.0		34.0	34.0		21.4	24.0		11.4	10.0
Cam		42.0	42.0		26.3	25.0	*	25.7	43.0		38.4	40.0		34.4	33.0	*	8.9	15.0
Sal		19.0	19.0		13.0	14.0	*	15.4	19.0		19.0	19.0		18.0	18.0		4.8	4.0
IseI	*	9.3	20.0		11.5	8.0	*	11.4	22.0		17.5	19.0		20.1	20.0		5.6	7.0
BuyI		24.0	24.0		17.2	16.0	*	18.0	23.0		24.0	24.0		18.0	21.0		7.1	7.0
Kib	*	9.3	16.0		12.7	13.0	*	11.3	22.0		23.0	23.0		16.4	15.0		7.1	9.0
MalN		19.0	19.0		9.4	10.0	*	12.7	19.0		17.1	19.0		15.4	15.0		5.6	6.0
MalW	*	19.5	23.0		15.5	17.0	*	15.0	24.0		24.0	24.0		24.0	24.0		10.3	7.0
MalO		20.3	22.0		20.3	20.0	*	15.5	23.0		24.0	24.0		24.0	24.0		8.7	8.0

* null alleles may be present at this locus, as is suggested by the generall excess of homozygotes for the most allele size classes

As evidence for null alleles was found at four markers and only six markers were currently available, all loci were included in the analyses. It was not possible to readily redesign new primers, thus the frequency for null alleles was statistically corrected (CHAKRABORTY *et al.* 1992, BROOKFIELD 1996). The frequencies of all alleles were re-estimated using the estimation "Brookfield 2" (BROOKFIELD 1996). This estimate takes also those individuals into account, which did not yield any amplification product ("null-homozygotes"). The amplification of several markers in two individuals of the first

population (Col) is probably due to a problem concerning the quality of the DNA. These individuals were consequently not taken into consideration in the re-estimation of the allele frequencies. The estimated frequency of the null alleles ranged from 0.12 (MalN) to 0.32 (Vih) for At-MS05, from 0.16 (Sal) to 0.33 (KiN) for At-MS58, from 0.076 (Cam) to 0.19 (IsiI) for At-MS93 and was 0.17 (Iku) for At-MS91.

MICRO-CHECKER (VAN OOSTERHOUT *et al.* 2004) allows an adjustment of genotypes according to null allele frequencies. These genotypes were used to generate a new matrix (Appendix Table A2). Based on the adjusted matrix several analyses were re-analysed and compared to the results of the original data set.

4.1.4. Allele Frequency Distribution

Five of six markers show a distribution with one or two common alleles of similar size and several alleles of low frequency at continuous lower and higher sizes (Appendix, Fig. A1). The expected stepwise increase of repeat units, reflected in an increasing allele size of two basepairs respectively, is interrupted sometimes by higher steps of more than two basepairs. The allele frequency distribution of At-MS93 indicates a bimodal distribution pattern. The smaller alleles occur at a size range between 283 and 297 bp, while the larger alleles occur between 327 and 341 bp. In-between a gap of 30 bp is presented where no alleles are found. Probably these differences in size are caused by an indel in the non-repetitive sequences. The allele distribution indicates that the mutational pattern of this marker does not follow a simple stepwise mutation model (KIMURA & OHTA 1978), which is predicted for microsatellites (ESTOUP & CORNUET1999). However, the mutation process of At-MS93 seems to be more complicated.

A geographic variation of allele frequencies can be examined at several markers (Fig. 19–24; Appendix Table A11–A16). The pattern is influenced either by the occurrence of private or rare alleles as well as by the proportion of allele frequencies. Private alleles at low frequency ($p \leq 0.1$) are found in several populations at each marker: Kib (At-MS05, At-MS58), KiS (At-MS42), Buk (At-MS42), Col (At-MS58, At-MS91), Cam (At-MS90) and KiWW (At-MS93). However the private allele ‘18’ of At-MS05 at the fragment Kaimosi exists at an intermediate frequency ($p = 0.18$). The special allelic composition of this population is also shown in the allele frequency distribution of At-MS91. An obvious geographic pattern at At-MS05 is also found between the northern and the southern populations. The allele ‘15’ occurs consequently in much higher proportion in the

investigated populations of the southern part of the continuous forest, while the same allele is rare in the populations of the northern part. The proportion of the allele ‘15’ in the fragment Kisere is between those of the southern and the northern forest. In slightly lower proportion it also occurs at two populations of Malawa. The most invariable loci, At-MS90, is monomorphic in the most populations.

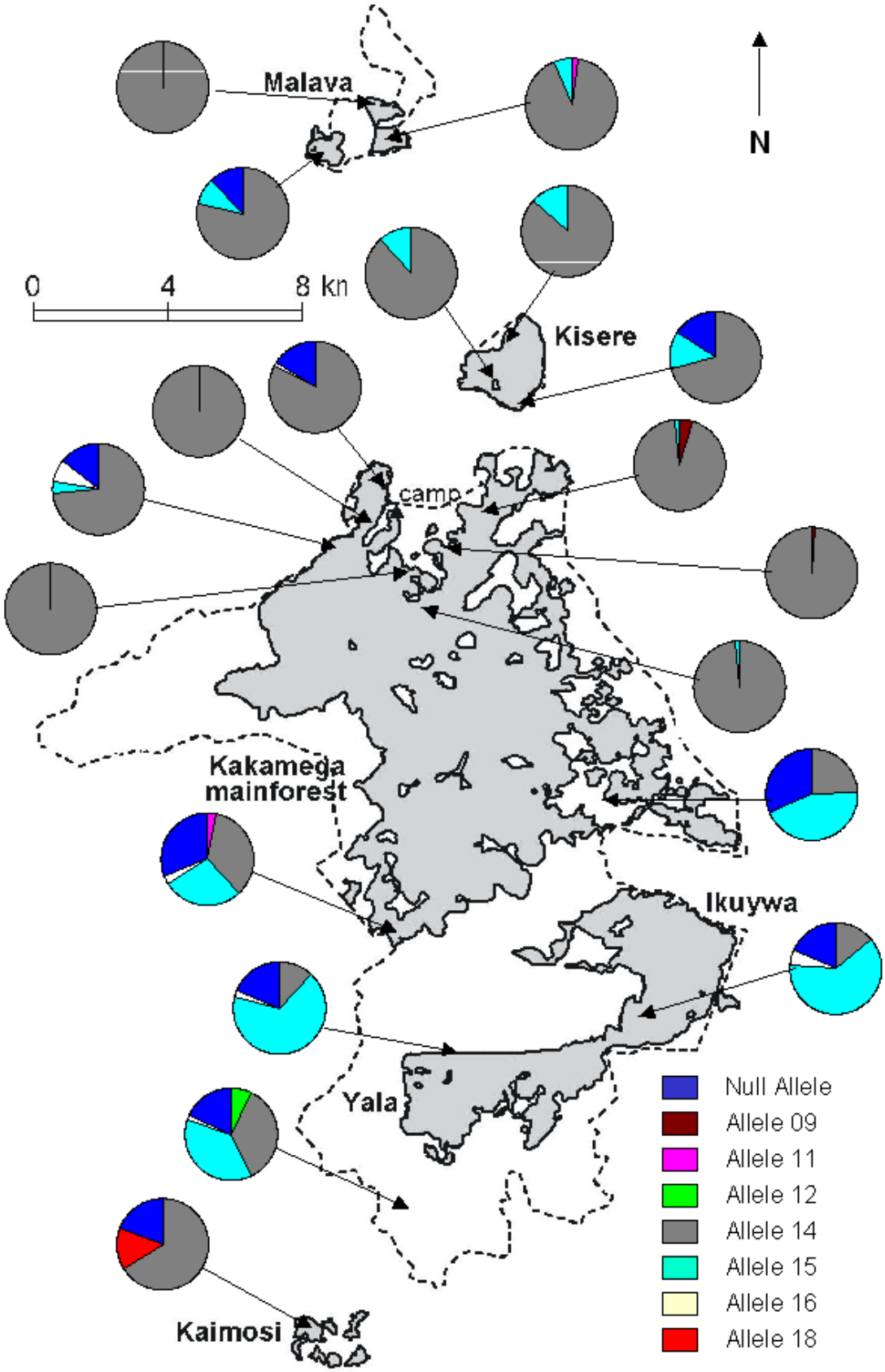

Fig. 19: Geographic variation of allele frequencies in the marker At-MS05 across 19 populations of *Amphitmetus transversus.*

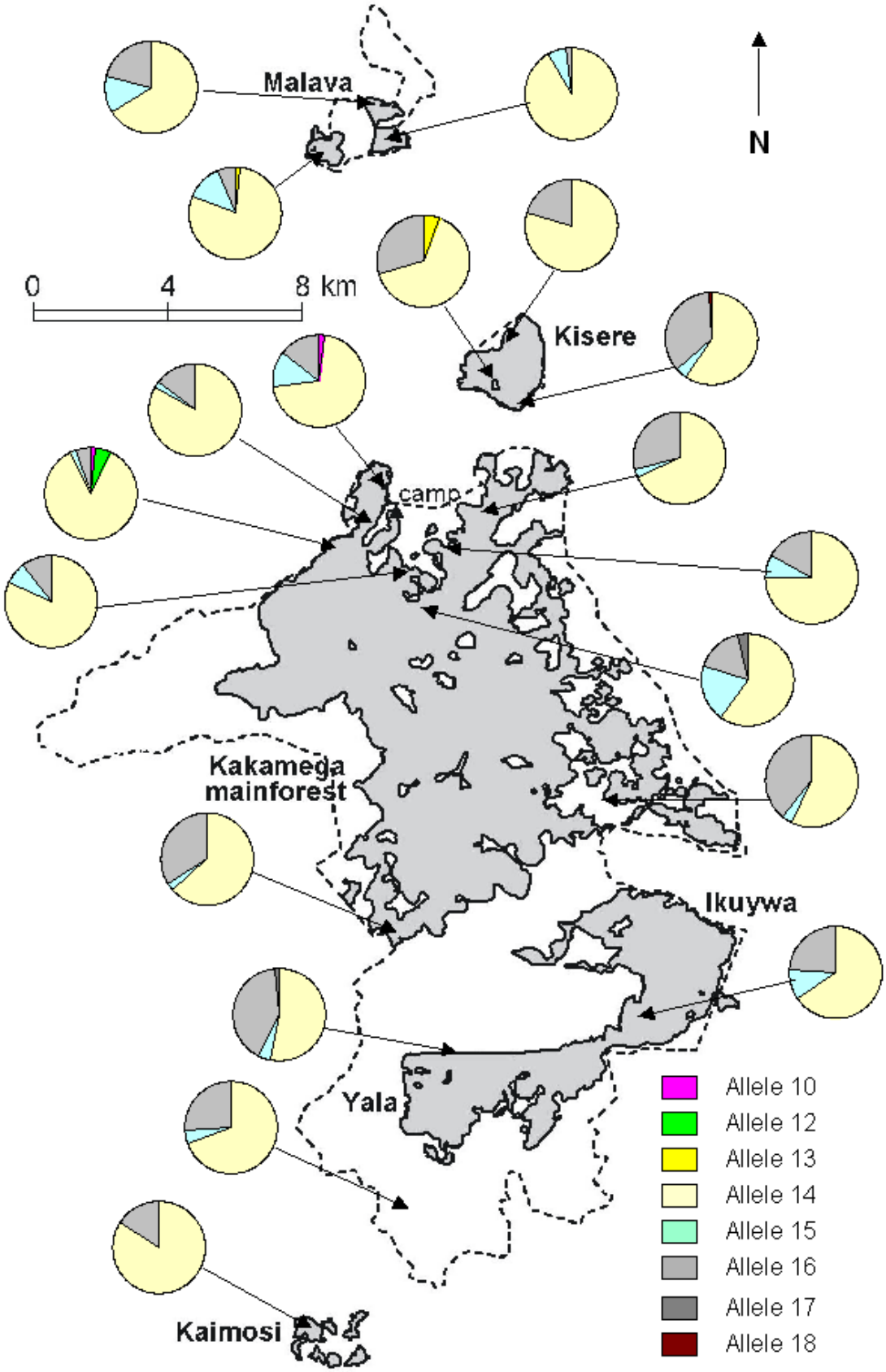

Fig. 20: Geographic variation of allele frequencies in the marker At-MS42 across 19 populations of *Amphitmetus transversus*.

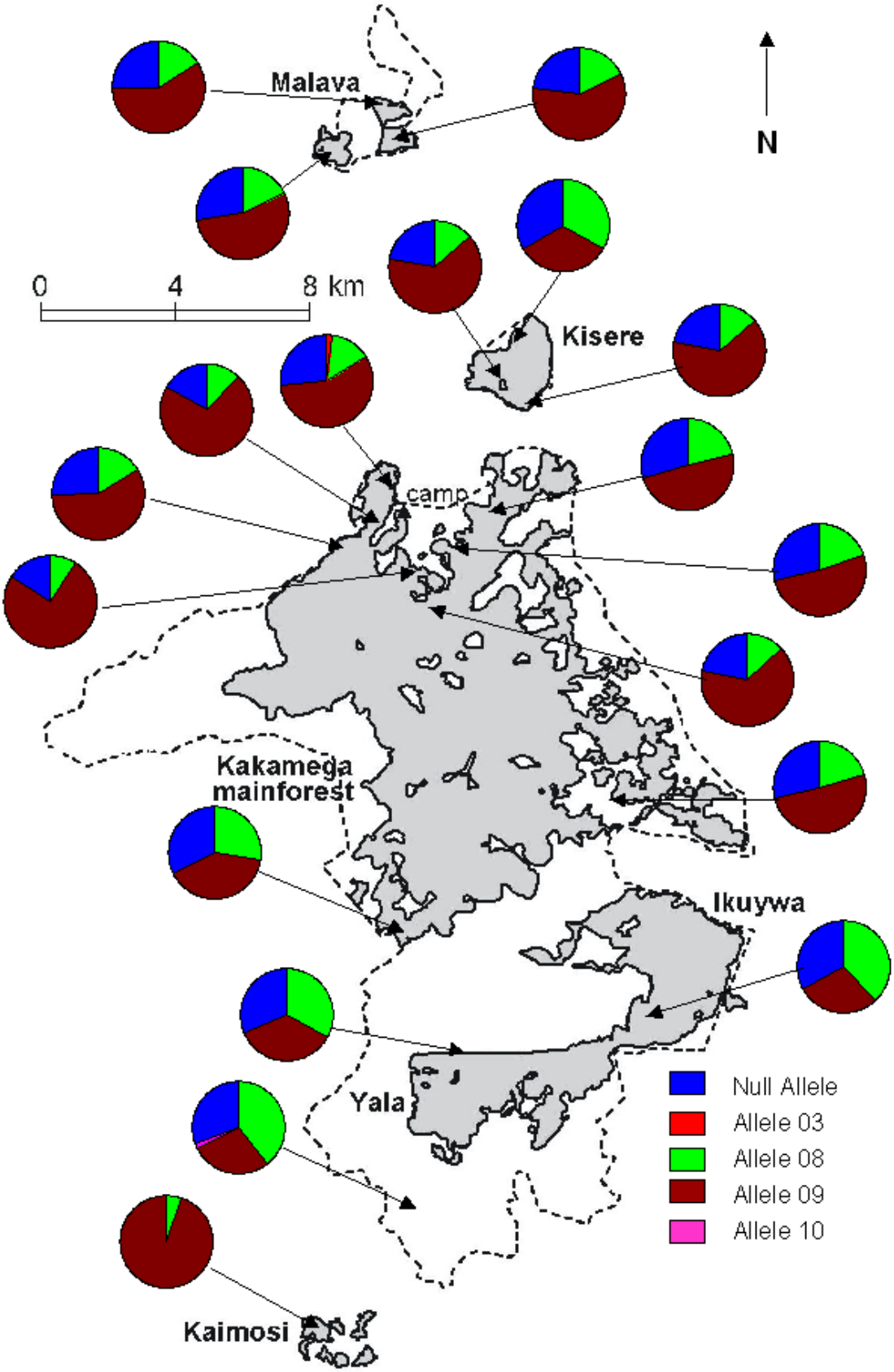

Fig. 21: Geographic variation of allele frequencies in the marker At-MS58 across 19 populations of *Amphitmetus transversus*.

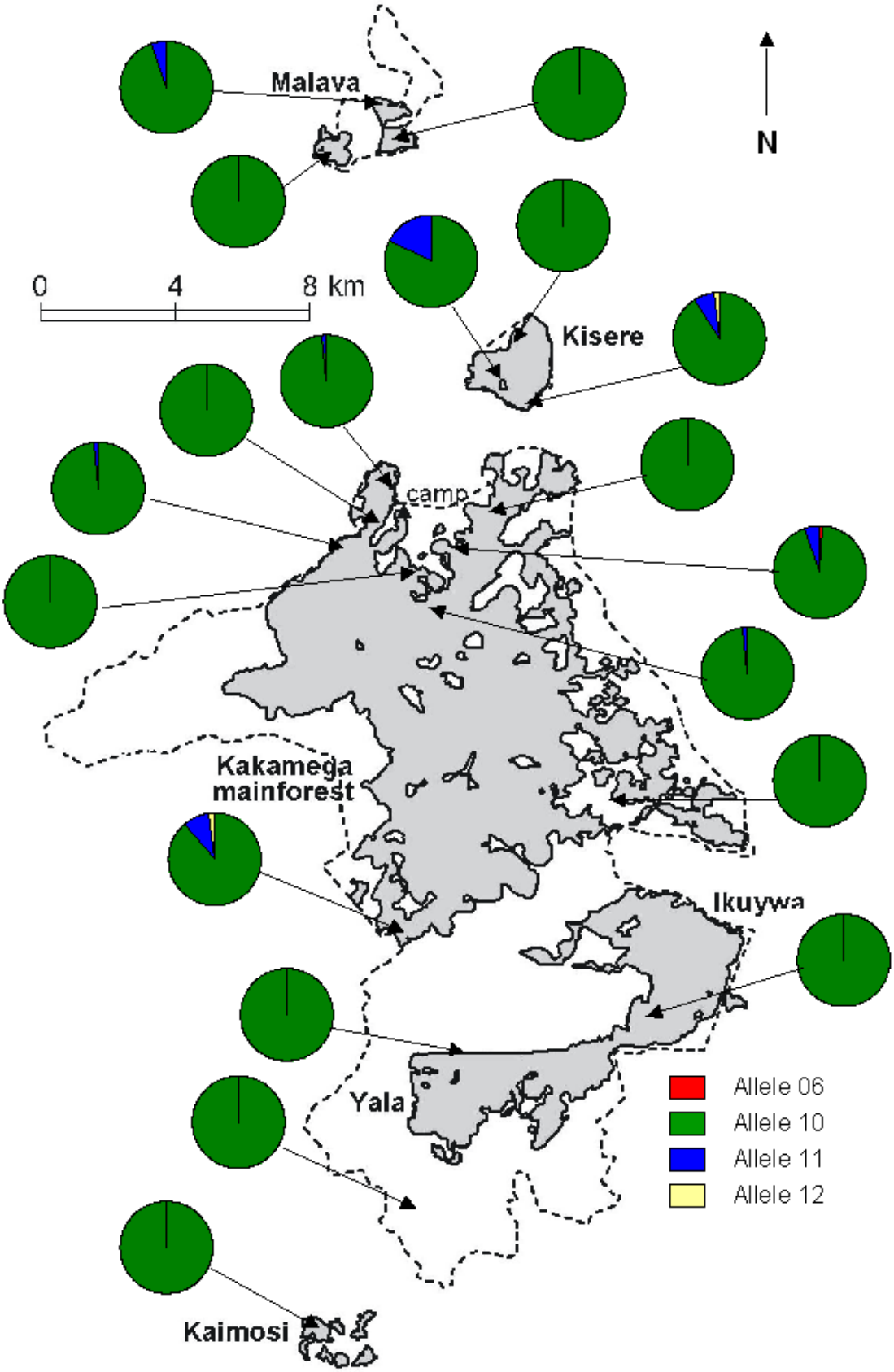

Fig. 22: Geographic variation of allele frequencies in the marker At-MS90 across 19 populations of *Amphitmetus transversus.*

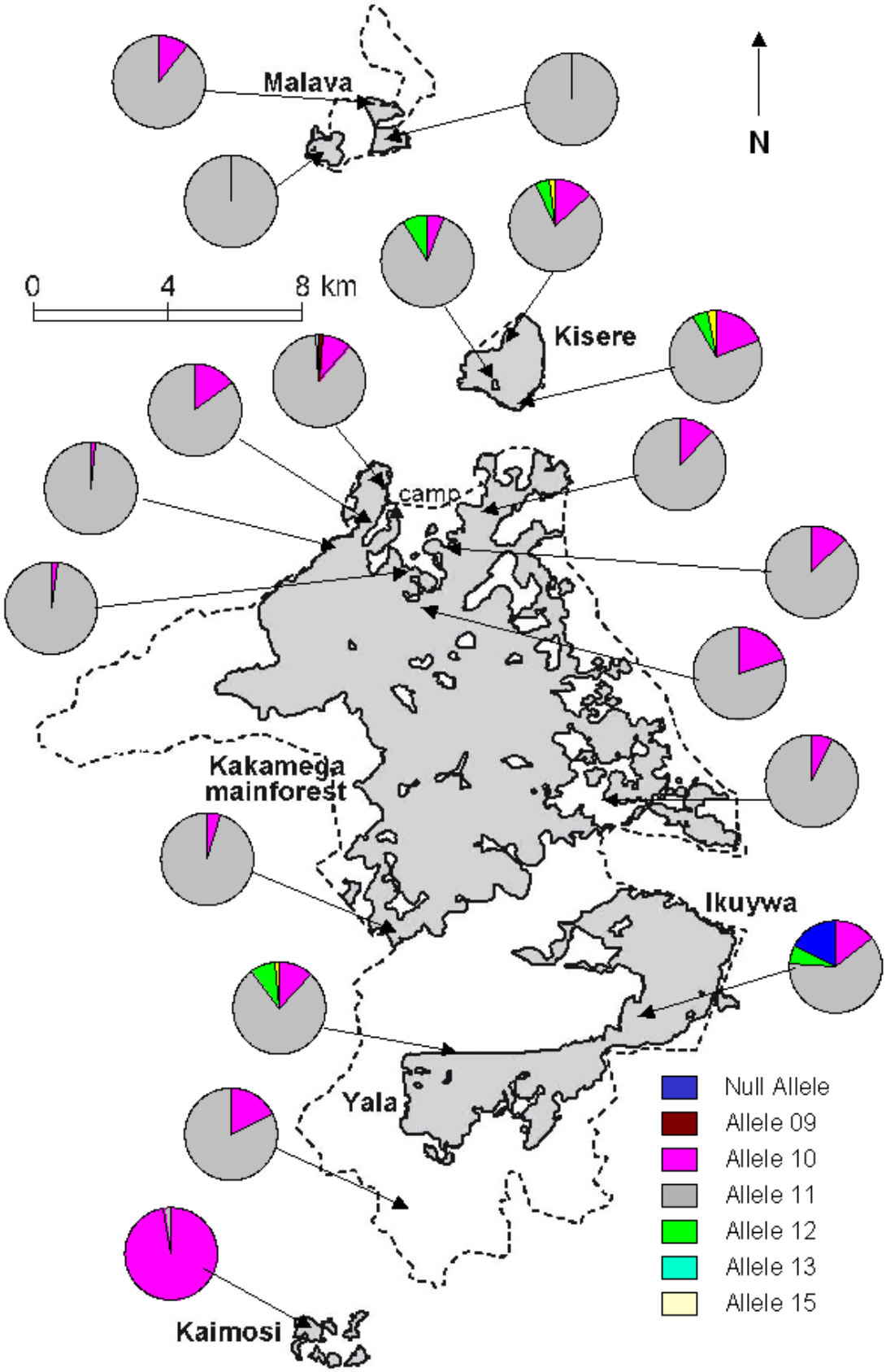

Fig. 23: Geographic variation of allele frequencies in the marker At-MS91 across 19 populations of *Amphitmetus transversus*.

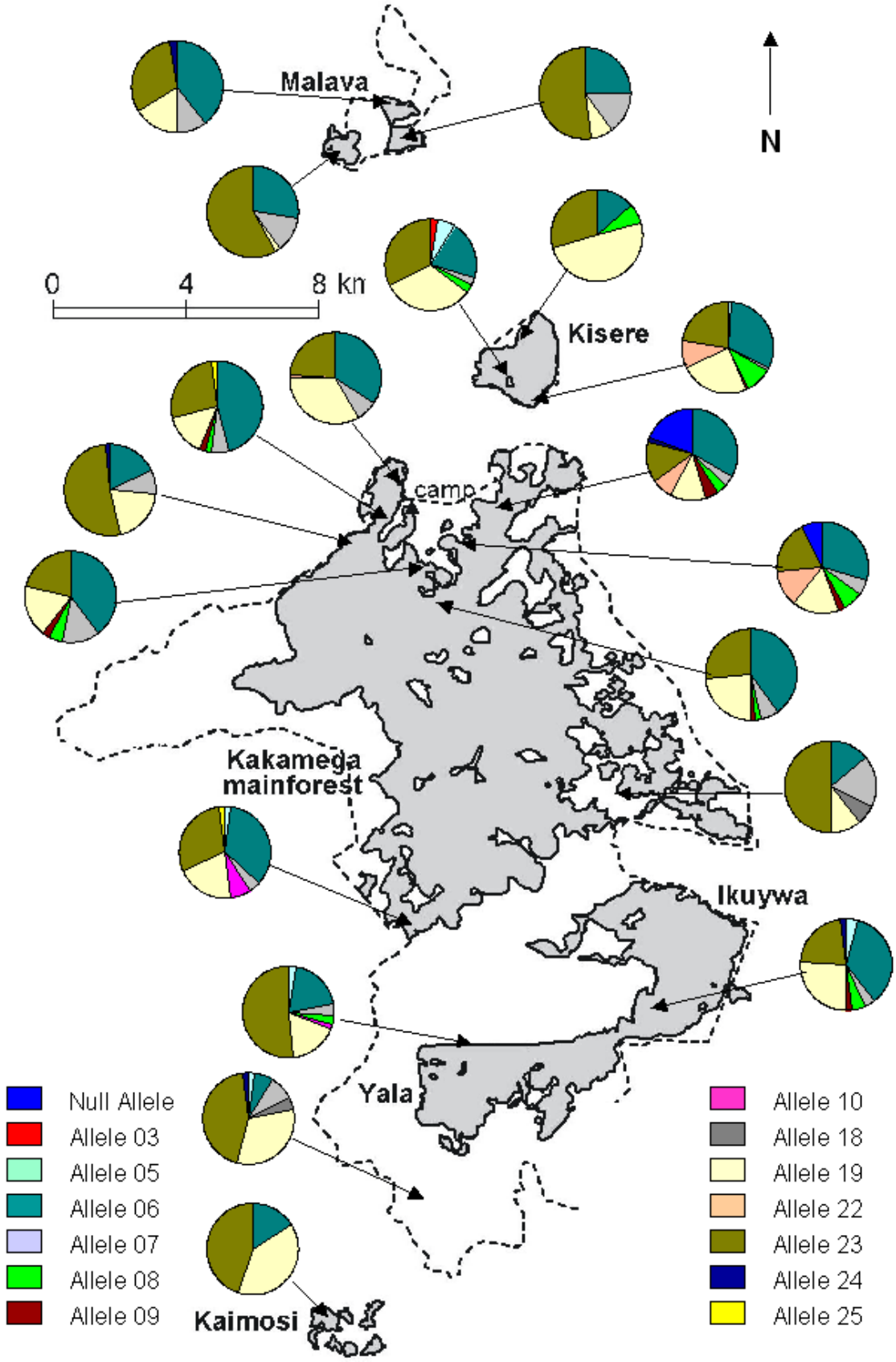

Fig. 24: Geographic variation of allele frequencies in the marker At-MS93 across 19 populations of *Amphitmetus transversus*.

4.1.5. Allelic Richness of the Populations

A test on logarithmic regression has shown a positive correlation between the number of sampled individuals and the mean number of alleles found within the 19 sample sites of *Amphitmetus transversus* (R^2 = 0.37; d.f. = 17 $p < 0.01$; Fig. 25). There is an asymptotic relationship between the number of alleles (A) and the number of sampled individuals within a population. A plateau is not yet reached. The test on linear regression was also significant (R^2 = 0.38., d.f. = 17 $p < 0.01$), which indicates that the relationship between N and the number of alleles is still nearly linear in the observed range and that effects of N on the number of alleles are great. Allelic richness (Â), inferred by the rarefaction method (Table 14), was tested on the number of sampled individuals. Rarefaction adjusted number of alleles to 14 individuals per populations. It showed no linear or rather logarithmic regression (linear regression: R^2 = 0.1, d.f. = 17 p = 0.186; logarithmic regression: R^2 = 0.1, d.f. = 17, p = 0.195; Fig. 26). Therefore Â can be treated as a measure of variability which is independent of sample size in the following analyses.

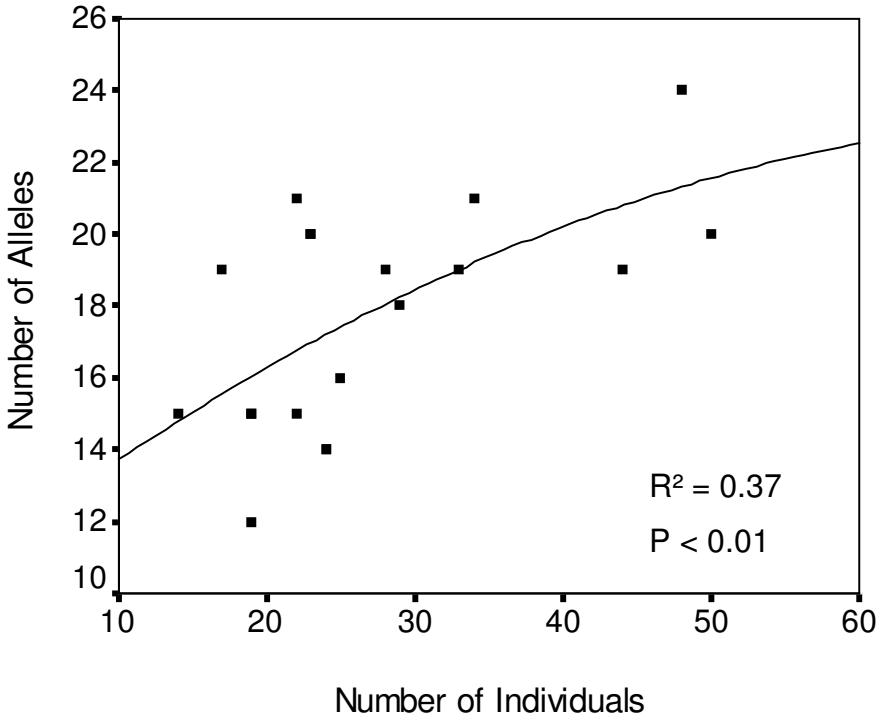

Fig. 25: Relationship between the number of alleles and the number of sampled individuals in *Amphitmetus transversus*. Number of alleles across all loci show a slightly asymptotic relationship to the number of sampled individuals (N = 19).

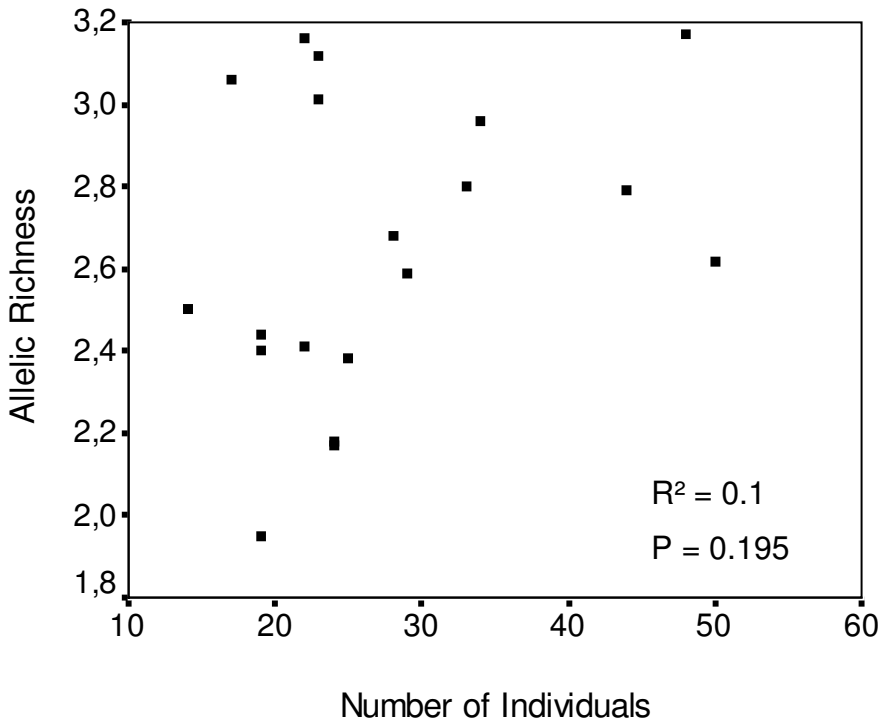

Fig. 26: Relationship between allelic richness (Â) and the number of sampled individuals in *Amphitmetus transversus*. Allelic richness is not correlated to the number of sampled individuals (N = 19).

Average allelic richness inferred from rarefaction reveals 1.95 (Kai) to 3.16 (IseI) alleles per site across all loci. Allelic richness per locus and population ranges from 1.00 to 6.92 alleles. The locus At-MS90 shows the smallest value of Â over all populations (Â = 1.40), while At-MS93 shows the highest value over all populations (Â = 5.21; Table 14).

A comparison of allelic richness between groups of populations reveals no significant difference in average allele numbers between fragmented (F) and continuous forest sites (CF) ($\hat{A}_{mean}$: F = 2.48 and CF = 2.75; P_{1000} = 0.14; Fig. 27). When fragments were divided into small (< 200 ha) and moderate (> 400 ha) categories there was a significant difference in allelic richness among the categories small fragments (SF; < 200 ha), moderate fragments (MF; > 400 ha) and continuous forest (CF) ($\hat{A}_{mean}$: CF = 2.75, MF: 2.88 and SF: 2.19; P_{1000} < 0.01). A single factor analysis of variance (ANOVA) yielded similar results ($F_{2,18}$ = 7.34; P < 0.01). A Bonferroni test of pairwise comparisons found significant differences between small fragments and continuous forest sites (P < 0.05), as well as between small fragment and moderate fragments (P < 0.01). No differences were found between moderate fragments and continuous forest sites (P = 1.0; Fig. 28).

The analyses were repeated with a matrix containing re-estimated null alleles. The results are similar to those obtained with the original data set. No significant differences in allelic richness between populations in the continuous forest and the fragments were found ($\hat{A}_{mean}$: F = 2.96 and CF = 3.05; P_{1000} = 0.74). However, when fragments were divided into small (< 200 ha) and moderate (> 400 ha) categories there was a significant difference in allelic richness ($\hat{A}_{mean}$: CF = 3.05, MF: 3.19 and SF: 2.43; P_{1000} < 0.01).

A Spearman-Rank-test showed a significant correlation between the average allelic richness (Â) and the size of the fragments and continuous forest respectively (N = 5, r = 0.9, p < 0.05), which implicits a positive relationship between fragment size and the number of alleles. The larger the area, the more alleles were found.

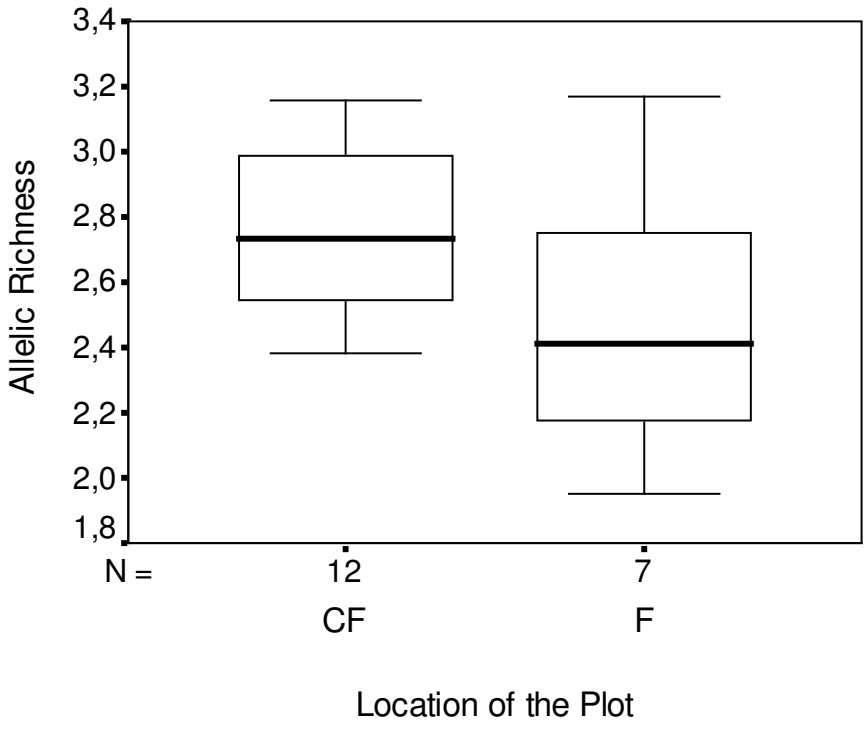

Fig. 27: Variation of allelic richness (Â) in *Amphitmetus transversus* among populations sampled in continuous forest (CF) vs. fragments (F) ($\hat{A}_{mean}$: F = 2.48 and CF = 2.75; P_{1000} = 0.14).

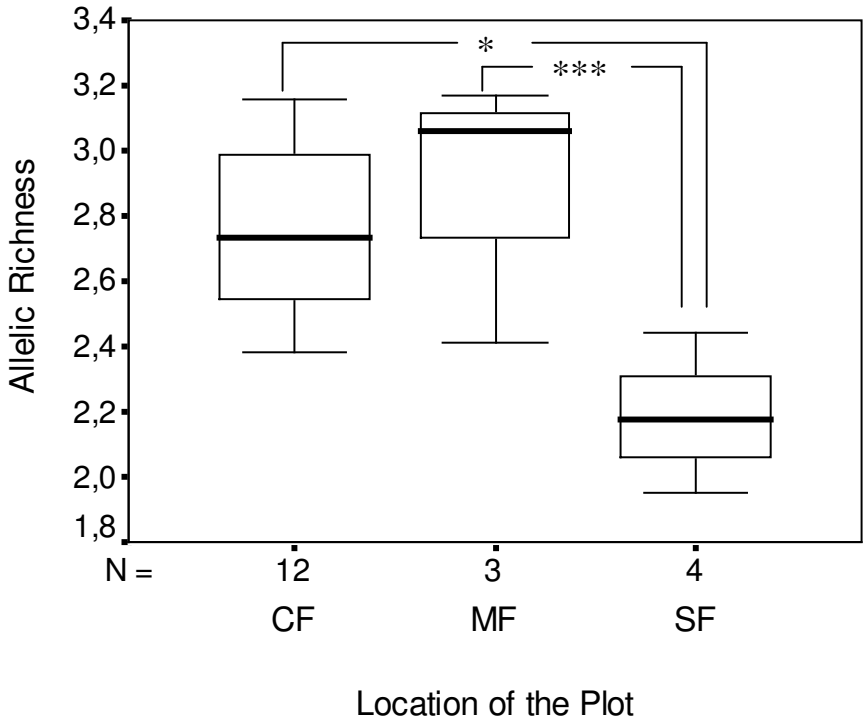

Fig. 28: Variation of allelic richness (Â) in *Amphitmetus transversus* among populations sampled in continuous forests (CF) vs. moderate fragments (MF; > 400 ha) vs. small fragments (SF; < 200 ha) ($Â_{mean}$: CF = 2.75, MF = 2.88 and SF = 2.19; $F_{2,18} = 7.34$; $P < 0.01$). Significant differences are indicated with an asterisk (*P < 0.05; **P < 0.01; ***P < 0.001).

Table 14: Allelic Richness per locus and population of *Amphitmetus transversus*. Results of rarefaction based on a min. sample size of 14 diploid individuals. Site codes follow Table 2.

Site	At-MS05	At-MS42	At-MS58	At-MS90	At-MS91	At-MS93	average
Col	1.49	3.48	2.50	1.49	2.56	4.21	2.62
BusI	1.47	3.72	2.00	1.47	2.00	4.86	2.59
Iku	2.95	2.99	2.00	1.00	2.98	6.77	3.12
Buk	2.86	3.76	2.00	1.50	1.50	4.47	2.68
IsiI	2.24	2.67	2.00	1.00	1.99	6.92	2.80
Vih	2.00	3.00	2.00	1.00	2.00	5.00	2.50
YalI	2.66	3.22	2.00	1.00	3.37	5.53	2.96
Cam	1.33	2.94	2.00	2.18	1.99	6.33	2.79
Sal	1.00	2.98	2.00	1.00	1.74	5.67	2.40
IseI	3.75	2.64	2.00	2.62	1.87	6.10	3.16
BuyI	1.00	2.58	2.00	1.00	2.00	5.68	2.38
Kib	3.59	2.85	2.61	1.00	2.00	6.00	3.01
MalN	1.00	3.00	2.00	1.94	2.00	4.73	2.44
MalW	1.99	3.51	2.00	1.00	1.00	3.58	2.18
MalO	2.52	2.52	2.00	1.00	1.00	3.97	2.17
KiN	2.00	2.00	2.00	1.00	3.51	3.96	2.41
KiWW	2.00	2.97	2.00	2.00	2.97	6.44	3.06
KiS	2.00	3.05	2.00	2.42	3.77	5.80	3.17
Kai	2.00	2.00	1.94	1.00	1.74	3.00	1.95
average	2.10	2.94	2.05	1.40	2.21	5.21	2.65

To test whether the differences of allelic variation between groups are evenly distributed across all markers or if single loci caused the observed pattern, tests were performed across groups of populations for each marker (Fig. 29). Data of the markers At-MS58, At-MS90 were not parametric and those were analysed using nonparametric tests. T-test for comparisons among continuous forest (CF) and fragments (F) showed no significant differences for At-MS05, At-MS42 and At-MS91, while significant differences between CF and F were found in At-MS93 ($T = 2.35$, d.f. = 17, $P < 0.05$). The nonparametric Mann-Whitney-Test among the mentioned categories did not reveal significant results for both At-MS58 and At-MS90 (Table 15).

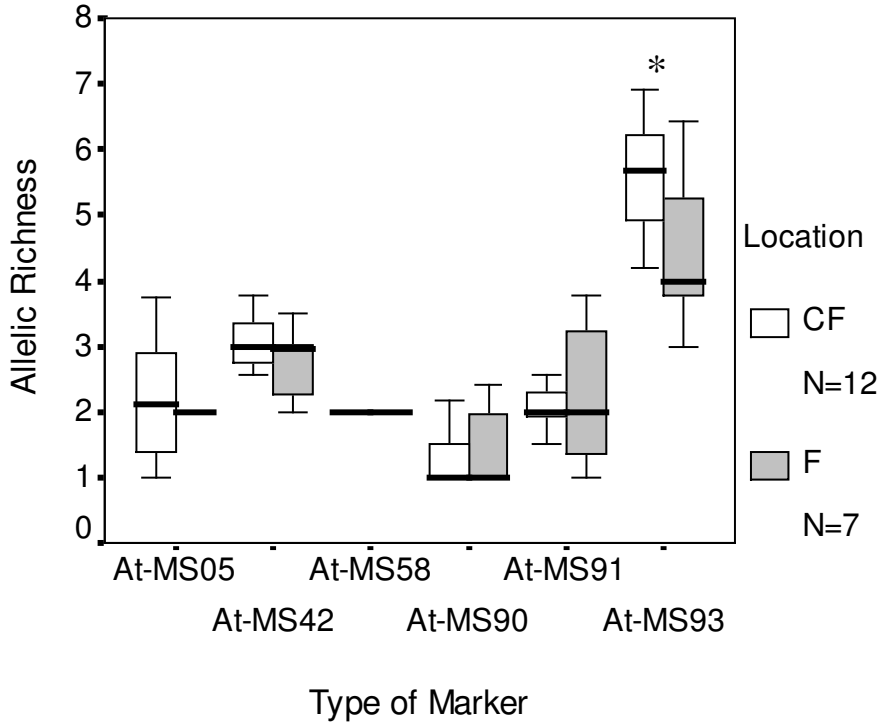

Fig. 29: Variation of allelic richness (Â) per marker in *Amphitmetus transversus* among populations sampled in continuous forest (CF) vs. fragments (F). Significant differences are indicated with an asterisk (*P < 0.05).

When fragments were divided into small (< 200 ha) and moderate (> 400 ha) categories there was a significant difference in allelic richness among the categories small fragment (SF; < 200 ha), moderate fragment (MF; > 400 ha) and continuous forest (CF) for At-MS91 ($F_{2,18} = 12.71$; $P < 0.001$) and At-MS93 ($F_{2,18} = 6.08$; $P < 0.05$). A Bonferroni test of pairwise comparisons showed significant differences between small fragments and moderate fragments ($P < 0.001$), as well as between continuous forest sites and moderate fragments ($P < 0.01$) in At-MS91. Tendencies for differences were found between small fragments and continuous forest sites ($P = 0.078$). In At-MS93 only differences between continuous forest sites and small fragments were significant ($P < 0.05$), while comparisons between continuous

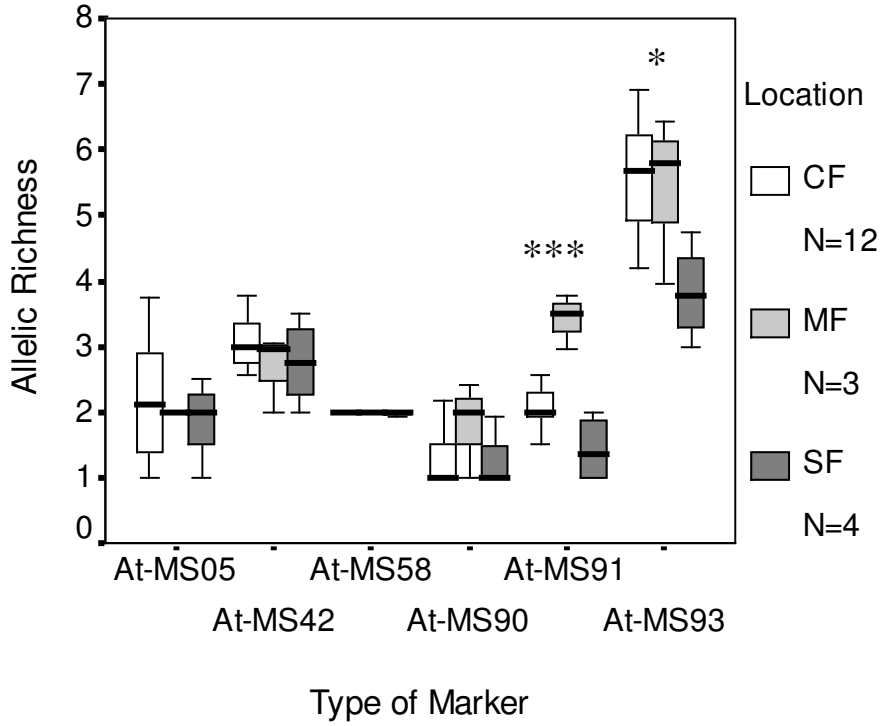

Fig. 30: Variation of allelic richness (Ä) per marker in *Amphitmetus transversus* among populations sampled in continuous forests (CF) vs. moderate fragments (MF; > 400 ha) vs. small fragments (SF; < 200 ha). Significant differences are indicated with an asterisk (*P < 0.05; **P < 0.01; ***P < 0.001).

Table 15: Summary of statistics for comparisons of allelic richness per marker among groups. T-test, ANOVA and Bonferroni Post-Hoc test were used for parametric data. Mann-Whitney and Kruskal-Wallis tests were used for nonparametric data. CF = continuous forest; MF = moderate fragment; SF = small fragments. Kolmogorov-Smirnov (KS) was used to test whether data are parametric.

	KS	CF-F	CF-MF-SF	CF-MF	CF-SF	MF-SF
At-MS05	Z = 0.78 P = 0.576	T = 0.68 d.f. = 17 P = 0.507	$F_{2,18}$ = 0.23 P = 0.794	P = 1.0	P = 1.0	P = 1.0
At-MS42	Z = 0.65 P = 0.799	T = 1.57 d.f. = 17 P = 0.136	$F_{2,18}$ = 1.18 P = 0.332	P = 0.661	P = 0.834	P = 1.0
At-MS58	Z = 2.25 P < 0.001***	Z = -1.60 d.f. = 17 P = 0.340	Chi² = 3.16 d.f. = 2 P = 0.207			
At-MS90	Z = 1.50 P < 0.05*	Z = -0.28 d.f. = 17 P = 0.837	Chi² = 1.81 d.f. = 2 P = 0.207			
At-MS91	Z = 1.26 P = 0.082	T = -0.31 d.f. = 17 P = 0.762	$F_{2,18}$ = 12.71*** P < 0.001	P < 0.001**	P = 0.078	P < 0.001***
At-MS93	Z = 0.60 P = 0.867	T = 2.354* d.f. = 17 P < 0.05	$F_{2,18}$ = 6.08* P < 0.05	P = 1.0	P < 0.05*	P = 0.108

* Significance at P < 0.05 , ** Significance at P < 0.01, *** Significance at P < 0.001

forest sites and moderate fragments as well as between moderate fragments and small fragments did not reveal significant differences. Neither At-MS05 and At-MS42 showed differences between the three groups nor At-MS58 and At-MS90 (Fig. 30).

4.1.6. Gene Diversity of the Populations

The gene diversity (H_S) per locus and site ranges from 0.000 to 0.806 and overall loci per site ranges from 0.223 (MalO) to 0.444 (IseI). The microsatellite marker At-MS90 shows the lowest H_S over all populations (H_S = 0.083), while At-MS93 obtains the highest H_S across all populations (H_S = 0.686; Table 16). There was no significant difference in average H_S per loci between fragments (range: 0.00–0.784 per locus) and continuous forest sites (range 0.00–0.806 per locus) found (H_{Smean}: CF = 0.342, F = 0.326; P_{1000} = 0.64; Fig. 31). When fragments were divided into small (< 200 ha) and moderate (> 400 ha) categories there was a significant difference in H_S among the categories small fragments (SF; < 200 ha; range H_S per locus: 0.000–0.728), moderate fragments (MF; > 400 ha; range H_S per locus: 0.000–0.784) and continuous forest (CF; range H_S per locus: 0.000–0.806) (P_{1000} < 0.05). To examine the differences between multiple groups an ANOVA including a Bonferroni Post-Hoc test was

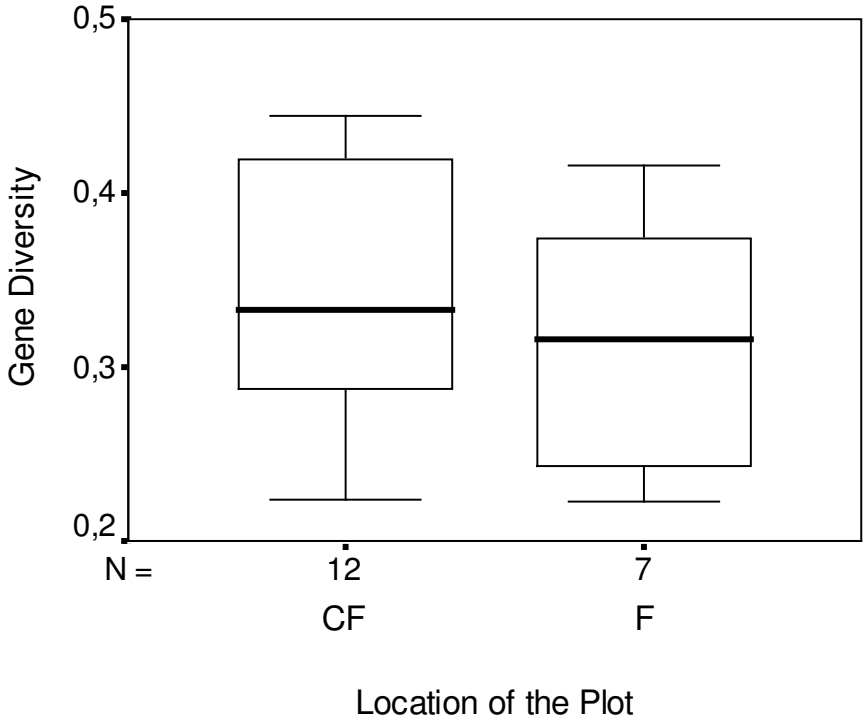

Fig. 31: Variation of gene diversity (H_S) across all loci among populations sampled in continuous forest (CF) vs. fragments (F) (H_{Smean}: CF = 0.342 and F = 0.326; P_{1000} = 0.644).

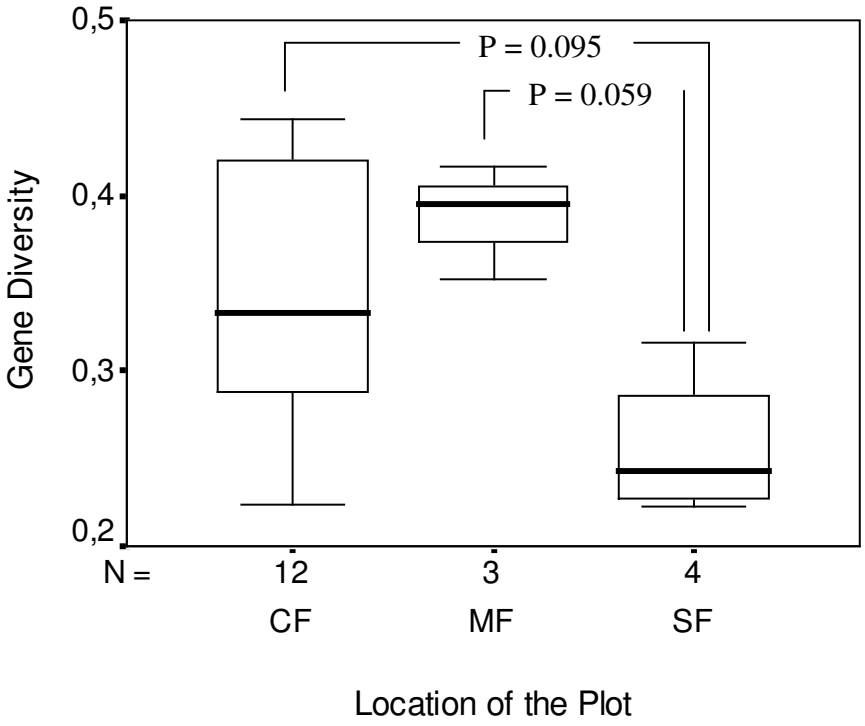

Fig. 32: Variation of gene diversity (H_S) across all loci among populations sampled in continuous forests (CF) vs. moderate fragments (MF; > 400 ha) vs. small fragments (SF; < 200 ha) (H_{Smean}: CF = 0.342; MF = 0.396; SF = 0.254; P1000 < 0.05).

Table 16: Gene diversity (NEI 1987) per locus and population of *Amphitmetus transversus*

Site	At-MS05	At-MS42	At-MS58	At-MS90	At-MS91	At-MS93	average
Col	0.041	0.466	0.357	0.040	0.226	0.716	0.308
BusI	0.033	0.582	0.287	0.033	0.324	0.722	0.330
Iku	0.401	0.514	0.514	0.000	0.431	0.776	0.439
Buk	0.263	0.263	0.349	0.036	0.036	0.663	0.268
IsiI	0.118	0.458	0.436	0.000	0.217	0.777	0.334
Vih	0.495	0.530	0.440	0.000	0.137	0.712	0.386
YalI	0.307	0.556	0.514	0.000	0.377	0.673	0.405
Cam	0.023	0.406	0.412	0.130	0.221	0.806	0.333
Sal	0.000	0.327	0.199	0.000	0.053	0.765	0.224
IseI	0.604	0.486	0.506	0.212	0.089	0.766	0.444
BuyI	0.000	0.289	0.259	0.000	0.257	0.705	0.252
Kib	0.615	0.457	0.532	0.000	0.292	0.708	0.434
MalN	0.000	0.520	0.351	0.105	0.193	0.728	0.316
MalW	0.194	0.362	0.391	0.000	0.000	0.580	0.255
MalO	0.160	0.159	0.368	0.000	0.000	0.651	0.223
KiN	0.245	0.331	0.524	0.000	0.354	0.655	0.352
KiWW	0.217	0.504	0.309	0.301	0.270	0.768	0.395
KiS	0.269	0.525	0.294	0.175	0.450	0.784	0.416
Kai	0.316	0.272	0.105	0.000	0.053	0.639	0.231
average	0.200	0.382	0.335	0.083	0.189	0.686	

conducted to correct for multiple comparisons ($F_{2,18}$ = 3.94; P < 0.05). The Bonferroni test revealed no significant differences between any pairs of groups, although differences between CF and SF (P = 0.095) as well as between MF and SF (P = 0.059) approached significance (Fig. 32).

An analysis on differences in gene diversity on the estimated null alleles yielded similar results. Differences between the continuous forest and the fragments were not significant (H_{Smean}: CF = 0.390, F = 0.436; P_{1000} = 0.35), while a test on differences between the three categories (continuous forest, moderate fragments and small fragments) gave a significant result (H_{Smean}: CF = 0.390; MF = 0.471; SF = 0.303; P_{1000} < 0.01).

To test whether the differences of gene diversity between groups are evenly distributed across all markers or if single loci cause the observed pattern, tests were performed across groups of populations for each marker (Table 17). No differences were found for any marker between the continuous forest (CF) and fragments (F) (Fig. 33).

Table 17: Summary of statistics for comparisons of gene diversity in *Amphitmetus transversus* per marker among groups. Parametric tests were used for normally distributed data (t-test; ANOVA, Bonferroni Post-Hoc). CF = continuous forest; MF = moderate fragment; SF = small fragments. Kolmogorov-Smirnov (KS) was used to test whether data are normally distributed.

	KS	CF-F	CF-MF-SF	CF-MF	CF-SF	MF-SF
At-MS05	Z = 0.62 P = 0.835	T = 0.43 d.f. = 17 P = 0.670	$F_{2,18}$ = 0.21 P = 0.816	P = 1.0	P = 1.0	P = 1.0
At-MS42	Z = 0.86 P = 0.456	T = 1.11 d.f. = 17 P = 0.281	$F_{2,18}$ = 1.68 P = 0.218	P = 1.0	P = 0.296	P = 0.519
At-MS58	Z = 0,56 P = 0.915	T = 1.19 d.f. = 17 P = 0.251	$F_{2,18}$ = 1.01 P = 0.386	P = 1.0	P = 0.523	P = 1.0
At-MS90	Z = 1.35 P = 0.054	T = -1.08 d.f. = 17 P = 0.295	$F_{2,18}$ = 3.05 P = 0.076	P = 0.098	P = 1.0	P = 0.138
At-MS91	Z = 0.55 P = 0.924	T = 0.48 d.f. = 17 P = 0.641	$F_{2,18}$ = 5.83* P < 0.05	P = 0.259	P = 0.086	P < 0.012*
At-MS93	Z = 0.71 P = 0.691	T = 1.69 d.f. = 17 P = 0.109	$F_{2,18}$ = 4.11* P < 0.05	P = 1.0	P < 0.05*	P = 0.134

* Significance at P < 0.05

When fragments were divided into small (< 200 ha) and moderate (> 400 ha) categories a significant difference in gene diversity among the categories small fragment (SF; < 200ha), moderate fragment (MF; > 400 ha) and continuous forest (CF) for At-MS91 ($F_{2,18}$ = 5.83; P < 0.05) and At-MS93 ($F_{2,18}$ = 4.11; P < 0.05) ocurred. A Bonferroni test of pairwise

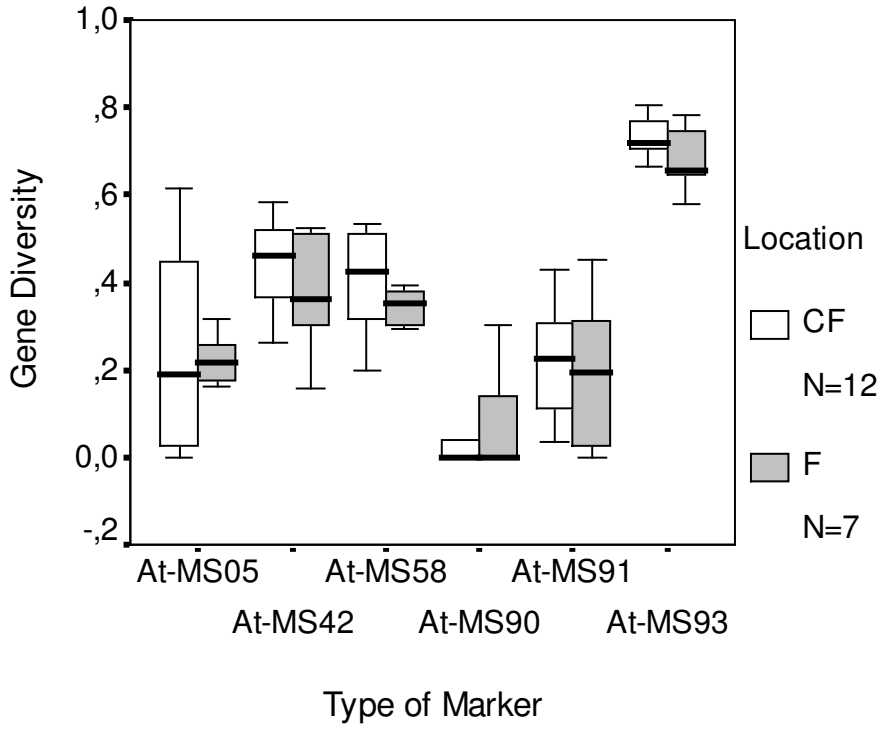

Fig. 33: Variation of gene diversity (H_S) per locus in *Amphitmetus transversus* among populations sampled in continuous forest (CF) vs. fragments (F).

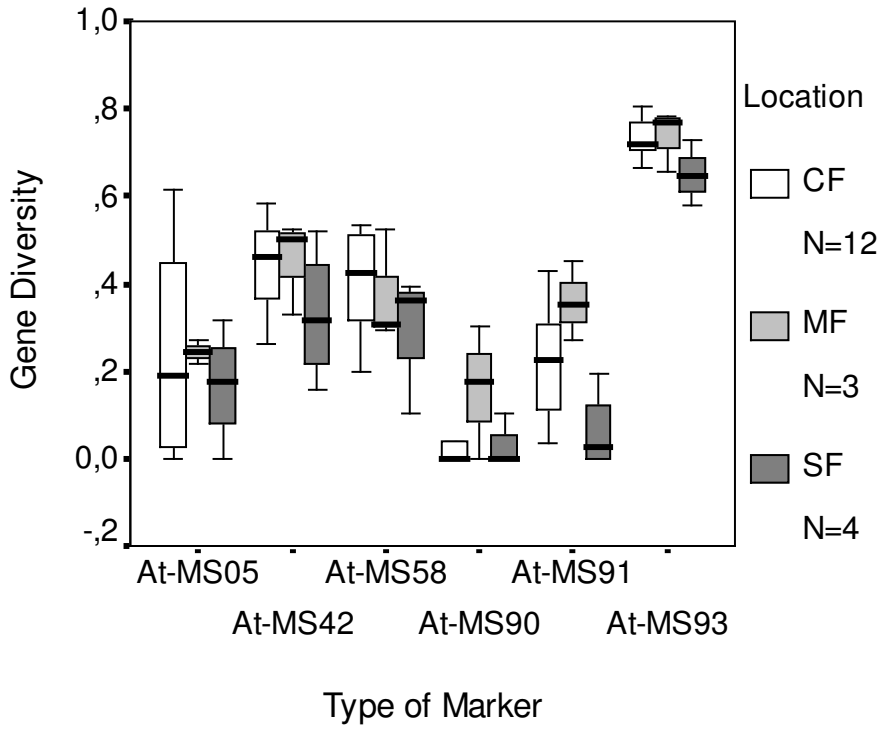

Fig. 34: Variation of gene diversity (H_S) per locus in *Amphitmetus transversus* among populations sampled in continuous forests (CF) vs. moderate fragments (MF; > 400 ha) vs. small fragments (SF; < 200 ha)

comparisons showed significant differences between continuous forest and small fragments ($P < 0.05$) at At-MS91, but differences between other forest types were not found. In At-MS93 only differences between continuous forest sites and small fragments were significant ($p < 0.05$). The other markers showed no differences at all (Fig. 34).

4.1.7. Allele Frequency Distribution and Mode-Shift

The proportion of alleles were grouped into ten allele frequency classes and then plotted in an allele frequency histogram for groups of populations located in the continuous forest and within fragments. Additionally, fragments were divided into small (SF; < 200 ha) and moderate categories (MF; > 400 ha) and plotted in a frequency histogram together with data from the continuous forest (CF). There was no significant modeshift in allele frequency distribution of population groups located in continuous forest (CF), fragments (F), moderate fragments (MF) as well as in small fragments (SF; Fig. 35, 36). However, the mean proportion of rare alleles tended to be lower in fragments than in continuous forest sites ($T = 1.82$, d.f. = 17, $P = 0.087$). As shown in an analysis of variance (ANOVA) there were no significant differences in the proportion of rare alleles between continuous forest sites, moderate fragments and small fragments ($F_{2,18} = 2.51$, $P = 0.113$), which could be verified in a Bonferroni test of pairwise comparisons. No significant differences between small fragments and continuous forest sites ($P = 0.675$), between small and moderate fragments ($P = 0.119$) as well as between moderate fragments and continuous forest sites ($P = 1.0$) were found.

Regarding the pattern of allele frequency distribution under consideration of allele frequencies of null alleles the differences of the frequency distribution of rare alleles between the different categories were similar to those mentioned before (Fig 37, 38). Tendencies for differences of the proportion of rare alleles between continuous forest and fragments could be found ($T = 1.79$, d.f. = 17, $P = 0.091$) but no significant differences between continuous forest sites, moderate fragments and small fragments.

However, considering the allele frequency distribution of single categories (Fig. 38) a mode-shift within the small fragments was observable. The proportion of the alleles at low frequency is equal to an intermediate frequency class.

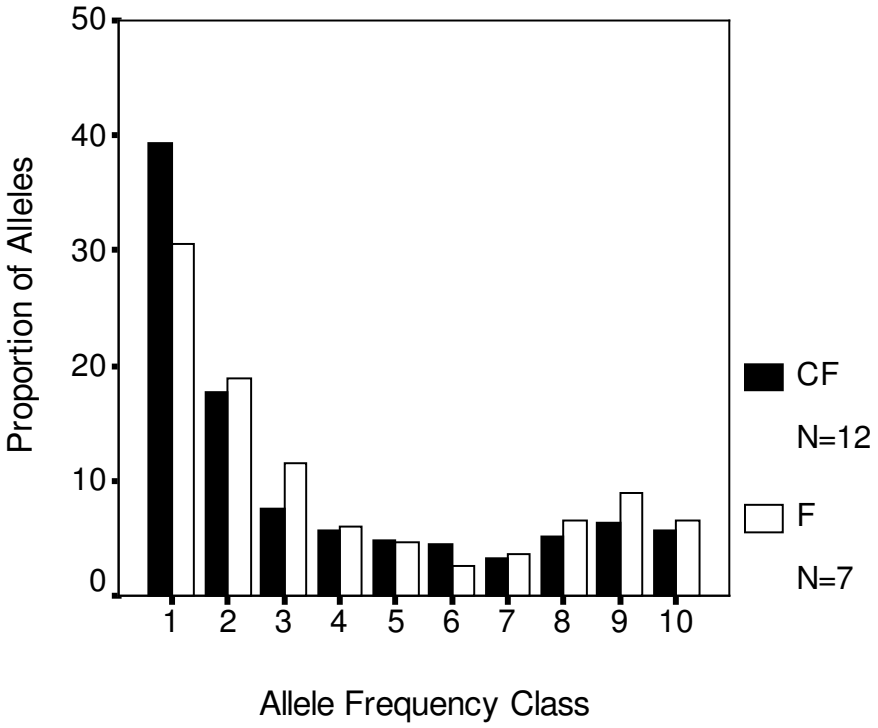

Fig. 35: Allele frequency distribution of populations of *Amphitmetus transversus* located within the continuous forest (CF) and fragments (F). Allele frequency classes according to LUIKART *et al.* (1998). The far left bar of each plot indicates the proportion of rare alleles (frequency less than 0.1).

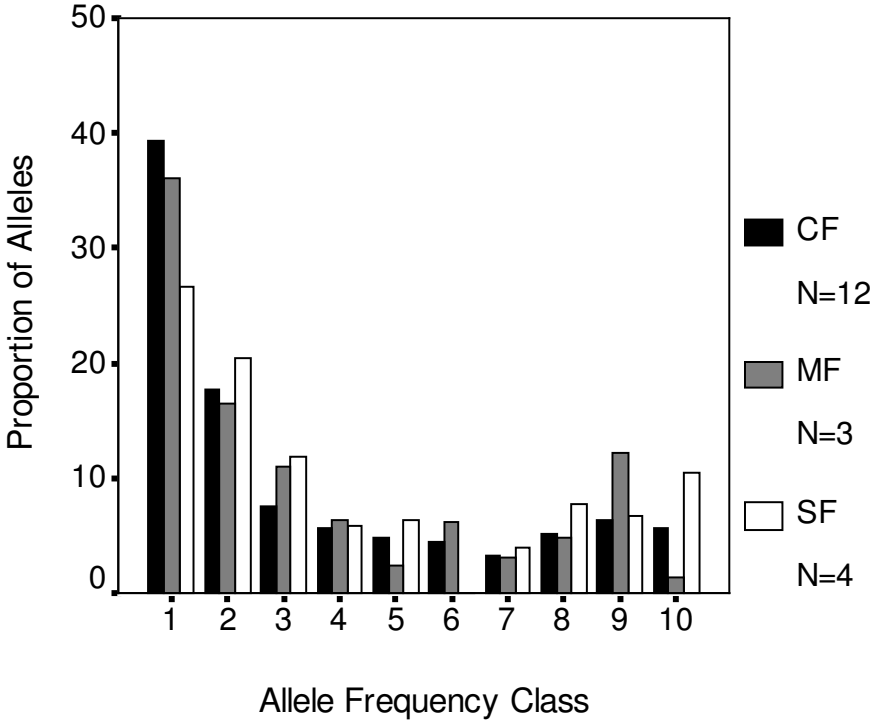

Fig. 36: Allele frequency distribution of populations of *Amphitmetus transversus* located within the continuous forest (CF), moderate fragments (MF) and small fragments (SF). Allele frequency classes according to LUIKART *et al.* (1998). The far left bar of each plot indicates the proportion of rare alleles (frequency less than 0.1).

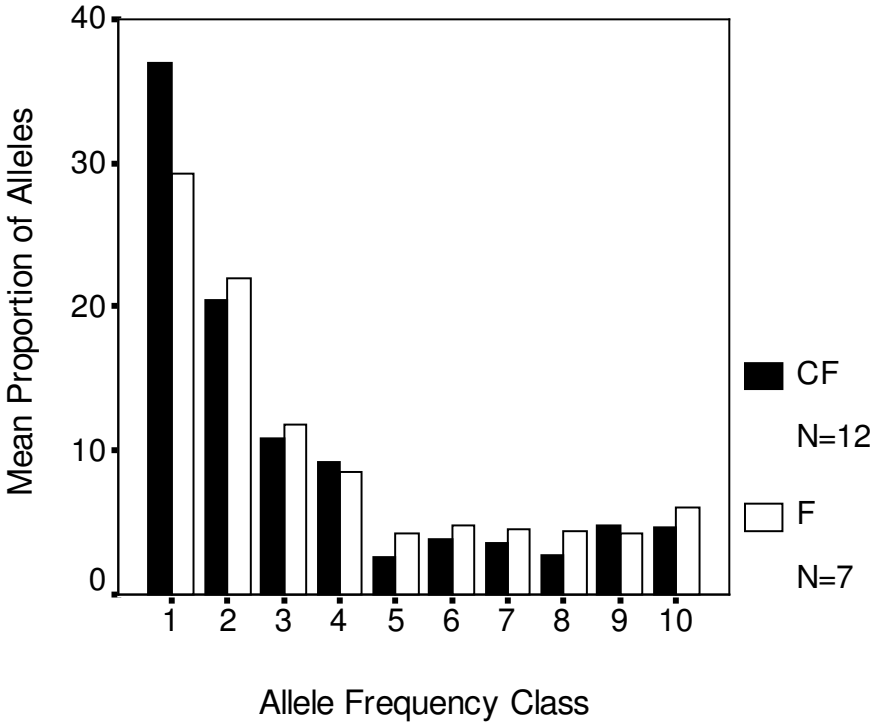

Fig. 37: Allele frequency distribution corrected for null alleles of populations of *Amphitmetus transversus* located within the continuous forest (CF) and fragments (F). Allele frequency classes according to LUIKART *et al.* (1998). The far left bar of each plot indicates the proportion of rare alleles (frequency less than 0.1).

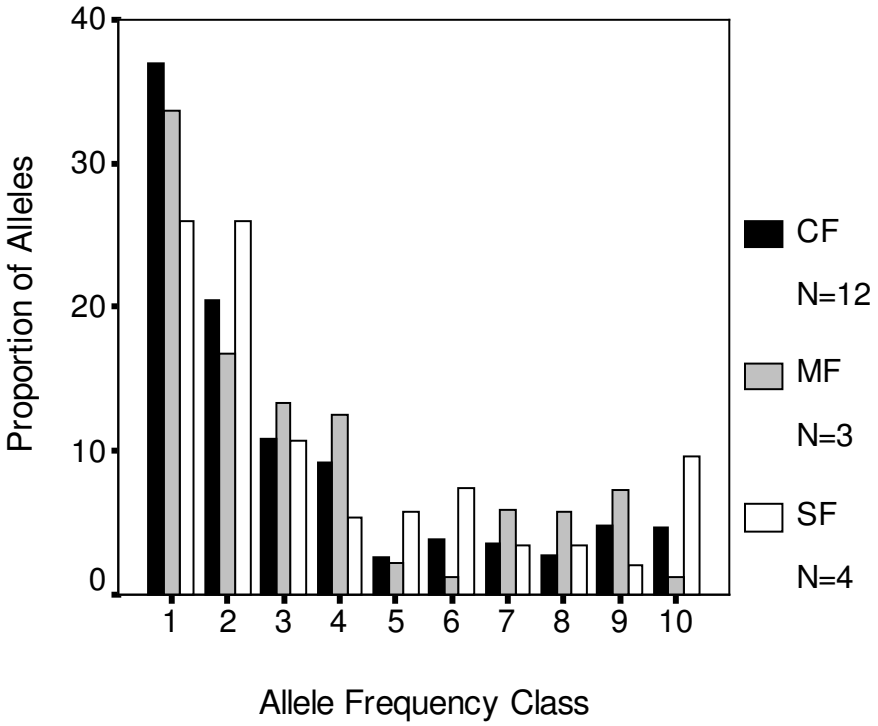

Fig. 38: Allele frequency distribution corrected for null alleles of populations of *Amphitmetus transversus* located within the continuous forest (CF), moderate fragments (MF) and small fragments (SF). Allele frequency classes according to LUIKART *et al.* (1998). The far left bar of each plot indicates the proportion of rare alleles (frequency less than 0.1).

4.1.8. Proportion of Polymorphic Loci

Three of six microsatellite markers in *Amphitmetus transversus* turned out to be monomorphic in at least one population. Therefore, the proportion of polymorphic loci was calculated for the categories continuous forest (CF), fragments (F), moderate fragments (MF) and small fragments (SF) (Table 18).

Table 18: Mean proportion of polymorphic loci (P_{mean}) within populations of the categories continuous forest (CF), fragment (F), moderate fragment (MF) and small fragment (SF). Standard deviation in brackets.

	CF (N = 12)	F (N = 7)	MF (N = 3)	SF (N = 4)
P_{mean}	0.875 (+/- 0.126)	0.833 (+/- 0.136)	0.944 (+/- 0.096)	0.750 (+/- 0.096)

Although fragments show a lower mean of polymorphic loci than continuous forest sites, there was no significant difference between the proportion of polymorphic loci in populations of continuous forest sites (CF) and fragments (F) found (T = 0.68, d.f. = 17, P = 0.508). Similar results are given focussing on the categories continuous forest (CF), moderate fragment (MF) and small fragment (SF). The differences are not significant as shown in an analysis of variance ($F_{2,18}$ = 2.64, P = 0.102). Corrections for multiple groups by a Bonferroni test confirm these results for pairwise comparisons (CF-MF; P = 1.0; CF-SF, P = 0.25; MF-SF, P = 0.14).

4.1.9. Test on Genetic Differentiation

The values of pairwise F_{ST} range between –0.0155 (MalO – MalW) and 0.4341 (MalO – Kai; Table 21). 129 of 171 pairwise distances were significant after sequential Bonferroni correction for multiple comparisons. A large proportion of non significant differences occurred within pairs of populations located in the northern part of the forest. Geographical distances between those pairs range from 0.74 km (KiWW–KiS) to 15.21 km (MalN–BusI). The great range indicates that only some of those similarities might be explained by geographically proximity, while others exhibit considerable similarities despite larger geographically distances. Very large genetic distances occur between the southern fragment Kaimosi and the remaining populations, whereas the greatest difference was found between

the population of the most southern fragment Kaimosi and one population of the most northern fragment Malawa (MalO).

Following the classification of WRIGHT (1978) the F-statistics indicate a moderate genetic differentiation among all populations (F_{ST} = 0.121; Table 19). Genetic differentiation across all populations was highly significant for each locus and over all loci regarding genic (Chi² = infinity, d.f. = 12, P < 0.001) as well as genotypic differentiation (Chi² = infinity, d.f. = 12, P < 0.001). The extent of genetic differentiation is mainly caused by two markers (At-MS05 and At-MS91), which show the greatest values for F_{ST} and indicate a genetic variation of 24.6 % in case of At-MS91 and 44.4% in case of At-MS05. In comparison the remaining four markers reveal a variance ranging between 4.3 % (At-MS93) to 7.5 % (At-MS58). The marker with the highest genetic variability (At-MS93) shows the smallest genetic differentiation across all populations.

The F_{IS} value over all populations is rather high, indicating a heterozygote deficiency found within single sample sites (see also global statistics of *Amphitmetus transversus*). High F_{IS} values are found for five of the six markers (At-MS05, At-MS58, At-MS90, At-MS91 and At-MS93), while At-MS42 exhibits a negative value indicating a heterozygote excess. As the high F_{IS} value is not consistent across all loci the result may be caused artificially by the presence of non-amplifying alleles. The highest value is given by At-MS58. This marker consists mainly of two different alleles which appear in the most of the analysed individuals in homozygote form. Only several individuals presented a heterozygous genotype.

Table 19: F-statistics of *A. transversus* according to WEIR & COCKERHAM (1984). Estimates were calculated by jack-knifing over loci (SE in brackets). Confidence interval (CI) was computed by bootstrapping (15000 times) over loci.

	Smallf (F_{IS})		Capf (F_{IT})		Theta (F_{ST})	
At-MS05	0.707	(+/– 0.048)	0.838	(+/– 0.047)	0.444	(+/– 0.115)
At-MS42	–0.059	(+/– 0.031)	–0.008	(+/– 0.032)	0.048	(+/– 0.014)
At-MS58	1.011	(+/– 0.016)	1.010	(+/– 0.015)	0.075	(+/– 0.028)
At-MS90	0.343	(+/– 0.069)	0.380	(+/– 0.066)	0.055	(+/– 0.022)
At-MS91	0.130	(+/– 0.070)	0.344	(+/– 0.168)	0.246	(+/– 0.184)
At-MS93	0.092	(+/– 0.037)	0.131	(+/– 0.033)	0.043	(+/– 0.009)
All:	0.268	(+/– 0.201)	0.363	(+/– 0.197)	0.121	(+/– 0.067)
95% CI	0.038–0.732		0.104–0.793		0.048–0.285	

The genetic differentiation between pairs of populations was also calculated on the re-estimated genotype matrix containing null alleles. The pairwise F_{ST} values range from –0.004

(MalO – MalW) and 0.405 (MalO – Kai). 132 of 171 pairwise distances were significant after a sequential Bonferroni correction.

The F-statistics, which were calculated on the information of the re-estimated genotype matrix containing null alleles, showed a similar result as the statistics on the original data set (Table 22). The extent of genetic differentiation across all populations, indicated as $F_{ST,}$ is slightly lower with a genetic variation of 10.7 % found between sample sites (Table 20). The F_{IS} values are much lower for the marker containing null alleles, because the re-estimation is based on the assumption of Hardy-Weinberg-Equilibrium, and hence, heterozygote deficits are much lower. The marker At-MS90 shows no evidence for null alleles and even no deviation from Hardy-Weinberg-Equilibrium, but the F_{IS} values are considerably high.

Table 20: F-statistics of *A. transversus* according to Weir & Cockerham (1984) on the re-estimated null allele matrix. Estimates were calculated by jack-knifing over loci (SE in brackets). Confidence interval (CI) was computed by bootstrapping (1500 times) over loci.

	Smallf (F_{IS})		Capf (F_{IT})		Theta (F_{ST})	
At-MS05	0.205	(+/– 0.039)	0.474	(+/– 0.073)	0.338	(+/– 0.083)
At-MS42	–0.059	(+/– 0.031)	–0.008	(+/– 0.032)	0.048	(+/– 0.014)
At-MS58	0.129	(+/– 0.018)	0.165	(+/– 0.021)	0.042	(+/– 0.017)
At-MS90	0.343	(+/– 0.069)	0.380	(+/– 0.066)	0.055	(+/– 0.022)
At-MS91	0.097	(+/– 0.056)	0.319	(+/– 0.162)	0.246	(+/– 0.175)
At-MS93	0.063	(+/– 0.024)	0.105	(+/– 0.020)	0.045	(+/– 0.009)
All:	0.079	(+/– 0.037)	0.179	(+/– 0.073)	0.107	(+/– 0.054)
95% CI	0.014–0.159		0.077–0.341		0.043–0.232	

4.1.10. Test on "Isolation by Distance"

The 'isolation by distance' pattern measured among all populations is highly significant ($P < 0.0001$; $F_{ST} = 0.011 + 8.97\ E^{-6}$ (distance); $R^2 = 0.38$; Fig. 39). The variance in F_{ST} among site pairs as well as average F_{ST} increases as a function of distance between populations. The increasing variance is caused by an increasing importance of drift relative to gene flow (HUTCHINSON & TEMPLETON 1999). The pattern of isolation by distance was also significant on the re-estimated genotype matrix containing null alleles ($P < 0.0001$; $F_{ST} = 0.013 + 7.78\ E^{-6}$ (distance); $R^2 = 0.23$).

Although the values obtained from analyses on the data set containing estimated null alleles are slightly different from those obtained from the original data set the general results derived from the corrected and the original data set were the same. It can be concluded that

null alleles do not bias the estimation of genetic differentiation significantly and therefore the analyses were continued on the original genotype matrix.

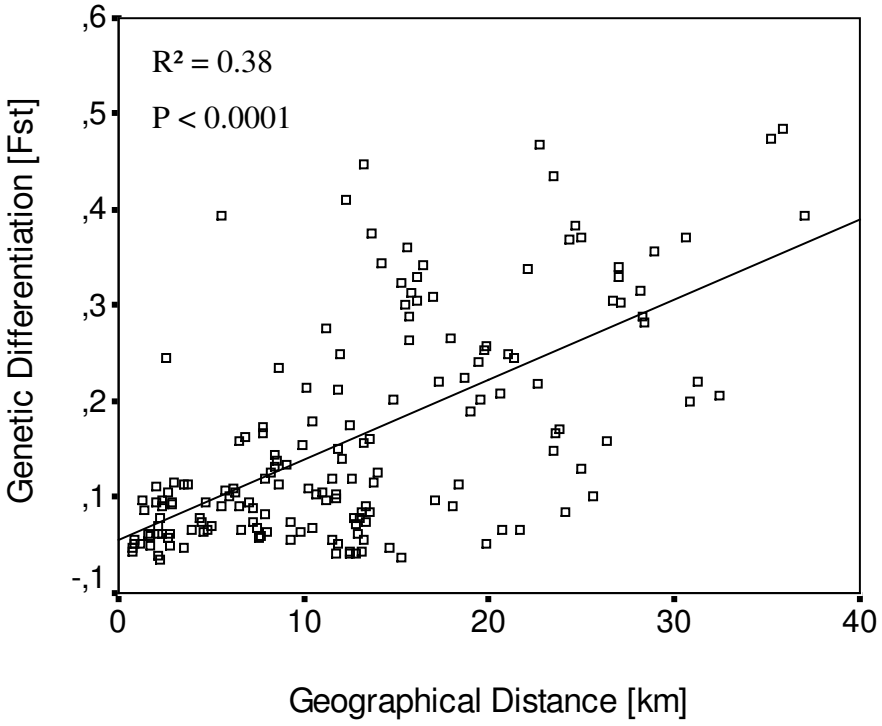

Fig. 39: Relationship between genetic (pairwise F_{ST}) and geographical distances in *Amphitmetus transversus* ($P < 0.0001$; $F_{ST} = 0.011 + 8.97\ E^{-6}$ (distance); $R^2 = 0.38$).

Table 21: Pairwise F_{ST} estimates across all loci on the upper matrix. The lower matrix indicates the significance for pairwise comparisons after sequencial Bonferroni correction: NS indicates no significant differences; *P < 0.05; **P < 0.01; ***P < 0.001. Site code follows Table 2.

Site	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalN	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO
Col		–0.0022	0.3218	0.2583	0.0408	0.0113	0.0597	0.0165	0.0285	0.1995	0.2924	0.0040	0.0030	0.1061	0.0066	0.1690	–0.0092	0.0593	0.0538
BusI	NS		0.2882	0.2440	0.0657	0.0122	0.0877	0.0243	0.0153	0.1852	0.2750	0.0117	0.0170	0.1056	0.0084	0.1713	–0.0138	0.0656	0.0756
Kai	***	***		0.3983	0.3859	0.3331	0.3215	0.3062	0.2319	0.4130	0.4178	0.3192	0.4188	0.3611	0.3490	0.3436	0.3432	0.4232	0.4341
Iku	***	***	***		0.2547	0.2146	0.1578	0.1917	0.1739	0.0513	0.0142	0.2383	0.2936	0.0445	0.2804	0.0326	0.2386	0.2531	0.2796
Buk	***	***	***	***		0.0635	0.0811	0.0391	0.0740	0.1631	0.2625	0.0445	0.0424	0.0995	0.0476	0.1403	0.0344	0.0009	–0.0093
IsiI	**	NS	***	***	***		0.0571	0.0247	0.0155	0.1644	0.2512	–0.0057	0.0209	0.0687	0.0095	0.1400	–0.0078	0.0640	0.0692
KiN	***	***	***	***	***	***		0.0461	0.0615	0.1521	0.2002	0.0544	0.1239	0.0639	0.1088	0.0519	0.0767	0.1163	0.1139
KiWW	***	***	***	***	**	***	NS		–0.0021	0.1115	0.2072	0.0201	0.0415	0.0463	0.0408	0.1088	0.0063	0.0639	0.0703
KiS	***	***	***	***	***	***	**	NS		0.1090	0.1945	0.0229	0.0473	0.0553	0.0343	0.1164	0.0143	0.0842	0.0933
Vih	***	***	***	NS	***	***	***	***	***		0.0132	0.1860	0.2330	0.0106	0.2256	0.0286	0.1679	0.1540	0.1944
YalN	***	***	***	NS	***	***	***	***	**	NS		0.2747	0.3245	0.0636	0.3117	0.0451	0.2653	0.2547	0.2892
Cam	NS	NS	***	***	***	NS	***	***	***	***	***		0.0116	0.0909	0.0080	0.1524	–0.0061	0.0523	0.0484
Sal	NS	NS	***	***	NS	*	***	*	**	***	***	NS		0.1299	-0.0099	0.2165	–0.0031	0.0550	0.0407
IseI	***	***	***	***	***	***	***	NS	***	NS	***	***	***		0.1243	0.0184	0.0807	0.0988	0.1211
BuyI	NS	NS	***	***	NS	NS	***	**	**	***	*	NS	NS	***		0.2041	–0.0071	0.0559	0.0458
Kib	***	***	***	NS	***	***	*	***	***	NS	**	***	***	NS	***		0.1553	0.1492	0.1701
MalN	NS	NS	***	***	NS	NS	***	NS	NS	***	***	NS	NS	***	NS	***		0.0277	0.0373
MalW	***	***	***	***	NS	***	***	***	***	***	***	***	NS	***	**	***	NS		– 0.0155
MalO	**	***	***	***	NS	***	***	***	***	***	***	***	NS	***	NS	***	NS	NS	

Table 22: Pairwise F_{ST} estimates based on matrix containing estimated null alleles across all loci in the upper matrix. The lower matrix indicates the significance of pairwise comarisons after sequential Bonferroni correction: NS indicates no significant differences; *P < 0.05; **P < 0.01; ***P < 0.001. Site code follows Table 2.

Site	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalN	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO
Col		–0.0027	0.3096	0.2164	0.0489	0.0183	0.0510	0.0140	0.0379	0.1843	0.2512	0.0076	0.0044	0.1234	0.0064	0.1536	–0.0084	0.0611	0.0478
BusI	NS		0.2781	0.1988	0.0692	0.0209	0.0724	0.0216	0.0268	0.1720	0.2344	0.0147	0.0176	0.1205	0.0082	0.1531	–0.0126	0.0655	0.0671
Kai	***	***		0.3136	0.3407	0.3253	0.2898	0.2889	0.2086	0.3527	0.3570	0.3093	0.3875	0.3272	0.3279	0.2963	0.3274	0.3787	0.4054
Iku	***	***	***		0.1820	0.1819	0.1149	0.1563	0.1072	0.0526	0.0204	0.1936	0.2502	0.0455	0.2353	0.0343	0.1980	0.1870	0.2376
Buk	***	***	***	***		0.0697	0.0566	0.0410	0.0612	0.1070	0.1880	0.0499	0.0564	0.0789	0.0584	0.0916	0.0438	0.0011	0.0050
IsiI	**	**	***	***	***		0.0541	0.0293	0.0284	0.1534	0.2143	–0.0021	0.0370	0.0948	0.0261	0.1311	0.0045	0.0680	0.0688
KiN	***	***	***	***	***	***		0.0339	0.0402	0.1143	0.1470	0.0452	0.1099	0.0657	0.0962	0.0438	0.0665	0.0888	0.0962
KiWW	***	***	***	***	**	***	NS		0.0072	0.1103	0.1743	0.0211	0.0384	0.0688	0.0368	0.0984	0.0058	0.0598	0.0634
KiS	***	***	***	***	***	***	NS	NS		0.0717	0.1326	0.0306	0.0588	0.0408	0.0461	0.0744	0.0256	0.0713	0.0895
Vih	***	***	***	NS	***	***	***	***	***		0.0164	0.1641	0.2176	0.0047	0.2100	0.0117	0.1564	0.1080	0.1797
YalN	***	***	***	NS	***	***	***	***	**	NS		0.2290	0.2839	0.0502	0.2709	0.0289	0.2256	0.1859	0.2467
Cam	NS	NS	***	***	***	NS	***	***	***	***	***		0.0234	0.1033	0.0176	0.1340	–0.0006	0.0527	0.0450
Sal	NS	NS	***	***	NS	*	***	*	**	***	***	NS		0.1491	–0.0098	0.1984	0.0010	0.0644	0.0409
IseI	***	***	***	***	***	***	***	NS	***	NS	***	***	***		0.1429	0.0174	0.1015	0.0871	0.1373
BuyI	NS	NS	***	***	**	NS	***	**	**	***	*	NS	NS	***		0.1870	–0.0045	0.0624	0.0429
Kib	***	***	***	*	***	***	*	***	***	NS	NS	***	***	NS	***		0.1427	0.1081	0.1548
MalN	NS	NS	***	***	NS	NS	***	NS	**	***	***	NS	NS	***	NS	***		0.0343	0.0336
MalW	***	***	***	***	NS	***	***	***	***	***	***	***	**	***	**	***	NS		–0.0044
MalO	**	***	***	***	NS	***	***	***	***	***	***	***	NS	***	NS	***	NS	NS	

4.1.11. Reasons of Genetic Differentiation - Partial Mantel Tests

Hypothesis I – Fragmentation: Following the results of the Mantel tests, fragmentation did not cause a significant genetic differentiation. Neither the simple nor the partial Mantel test confirmed the hypothesis (Table 23). The fragmentation might be not long enough to cause a significant differentiation due to limited gene flow. It is also possible that other reasons for genetic differentiation cover smaller effects caused by the fragmentation event. Nevertheless, at the present state there is no evidence for an increased genetic differentiation due to anthropogenic habitat fragmentation.

Hypothesis II – Riverine barriers: The simple Mantel test revealed a significant genetic differentiation due to the separation by the two major rivers of the Kakamega Forest (Table 23). Pairs of populations separated by one or two rivers are genetically more distant than those inhabiting an undivided area. This indicates that the concerned rivers act as barriers to gene flow and prevent migration between separated sites. However, the partial Mantel test, which accounts for the effects of geographical distance, was not significant. Hence, the significant result of the simple Mantel test might be influenced by the effect of an 'isolation by distance' pattern. A partial Mantel test for geographical distance when controlling for riverine barriers was not significant. It can be concluded that geographical distance did not act as genetic barrier within the sub-areas in-between the riverine barriers (Fig. 40).

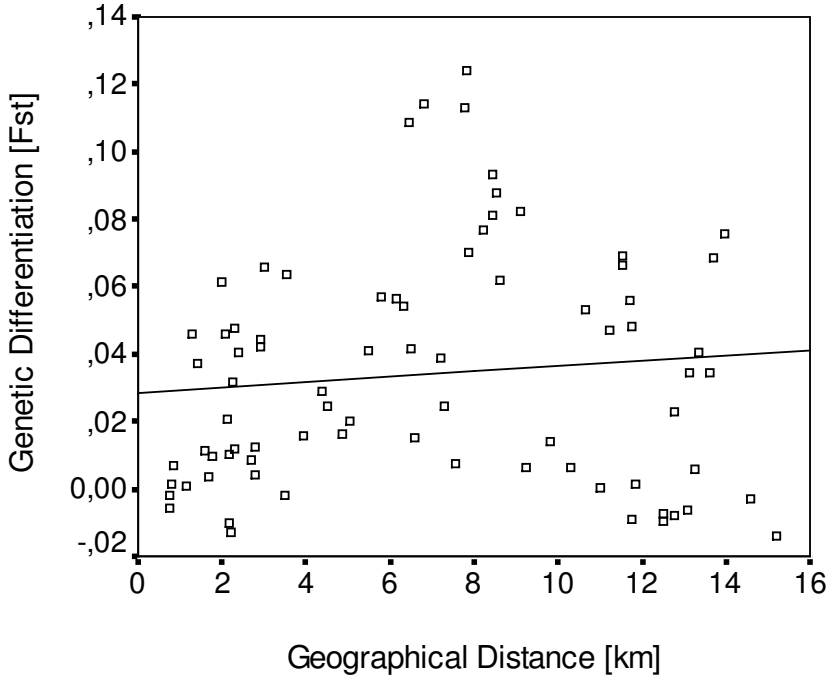

Fig. 40: Relationship between genetic (pairwise F_{ST}) and geographical distances for 13 populations of *Amphitmetus transversus* located in the northern part of Kakamega Forest (P = 0.067; R_{ST} = 0.029 + 1.24 E^{-6} (distance), R^2 = 0.010).

Hypothesis III – Ecological differentiation: The hypothesis of genetic differentiation due to different vegetation types was confirmed in a simple but not in a partial Mantel test under control of the geographic distances between populations (Table 23). However, an analysis of the effect of geographical distance on genetic distance under control of the effect of the ecological differentiation was not significant, too. The genetic differentiation on differences in soil structure was tested in a binary matrix. The model was significant in a simple Mantel test, but the significance was not maintained in a partial Mantel test under the control of geographical distance. The effect of isolation by distance was still significant in a partial Mantel test under the control of the matrix reflecting the soil structure of the Kakamega Forest.

Table 23: Results of simple and partial Mantel tests after 10000 permutations investigating the relationship between matrices of genetic distances and fragmentation, riverine barriers, vegetation type and soil structure. Level of significance was adjusted by a sequential Bonferroni correction for multiple tests.

Mantel test	P-value	significance	g
Genetic distance x geographic distance	0.00	***	0.654
Genetic distance x fragmentation	53.75	NS	0.112
Genetic distance x riverine barriers	0.00	***	0.622
Genetic distance x vegetation type	0.02	*	0.781
Genetic distance x soil structure	0.00	***	0.378
(Genetic distance x riverine barriers). geographical distance	11.55	NS	0.320
(Genetic distance x vegetation type). geographical distance	0.14	NS	0.688
(Genetic distance x soil structure). geographical distance	81.26	NS	0.029
(Genetic distance x geographical distance). riverine barriers	1.87	NS	0.428
(Genetic distance x geographical distance). vegetation type	52.34	NS	0.120
(Genetic distance x geographical distance). soil structure	0.02	*	0.638

*** significance at $P < 0.001$, NS = no significance

4.1.12. The Detection of Genetic Barriers

The first barrier, defined on a Monmonier's maximum difference algorithm, separates the population of the southern fragment Kaimosi from all other populations (Fig. 41). The thickness of the barrier is plotted proportionally to the frequency of achieving the given result during the jack-knife analysis based on 50 matrices. The barrier between Kaimosi and the remaining populations was obtained in each of the analysis (100 %). The distance between Kaimosi and the adjacent population (Kib) is about D = 0.344. Another barrier was detected splitting the southern part of the forest from the northern part. The two barriers are based on genetic differences between BusI and IseI (D = 0.106) as well as between BusI and Vih (D = 0.185). The first was obtained in 82 % of the cases while the latter appeared in 88 % of the analyses. Several smaller barriers, which occurred during the analyses of the multiple matrices, were not significant as the thin pink lines and the adjacent numbers indicate. One of these smaller barriers separates the population KiN from the surrounding populations. The result of the analysis based on multiple matrices is similar to that obtained by using the original matrix. The first separation is drawn between the southern fragment Kaimosi and the other populations, while the second barrier is located between the northern and the southern part of the forest.

The result was confirmed in defining groups of populations with a simulated annealing approach implemented in the program SAMOVA (DUPANLOUP *et al.* 2002) for two but not for three groups (Table 24). The final configuration in consideration of two groups revealed a separation between the southern fragment Kaimosi and the other populations similarly to the result of the Monmonier's maximum difference algorithm. In this constellation the maximum of about 23.8 % of the genetic variance is distributed among the groups while approximately 11.4 % of variation is left within single groups. Regarding three groups, Kaimosi is separated from all other populations. However, the southern populations Yala I and Ikuywa form a configuration separated from the remaining sample sites. The proportion of genetic variance across the three defined groups is lower than for the composition of two groups. In total 22.5 % of the genetic variance is found among the groups. Furthermore, only 6.5 % of genetic variation is left within single groups.

Table 24: Fixation indices corresponding to the population groups as inferred by SAMOVA algorithms for *Amphitmetus transversus* populations

		Group composition	F_{SC}	F_{ST}	F_{CT}
Two groups	1.	Kai	0.114***	0.325***	0.238***
	2.	other populations			
Three groups	1.	Kai	0.065***	0.276***	0.225***
	2.	Yalı & Iku			
	3.	other populations			

***P<0.001

Following the classical relationship $(1-F_{ST}) = (1-F_{SC})(1-F_{CT})$ an increase of the observed number of groups (K) is expected to contribute to the distribution of genetic variance within and among the defined groups. While F_{CT} is expected to increase with K, F_{SC} is expected to decrease (DUPANLOUP *et al*. 2002). Following DUPANLOUP *et al.* (2002) this assumption can be used in order to find the correct number of groups, because the largest mean F_{CT} value should be associated with the real number of groups. Under this presumption the separation of the 19 populations into two clusters seems to be more realistic than into three.

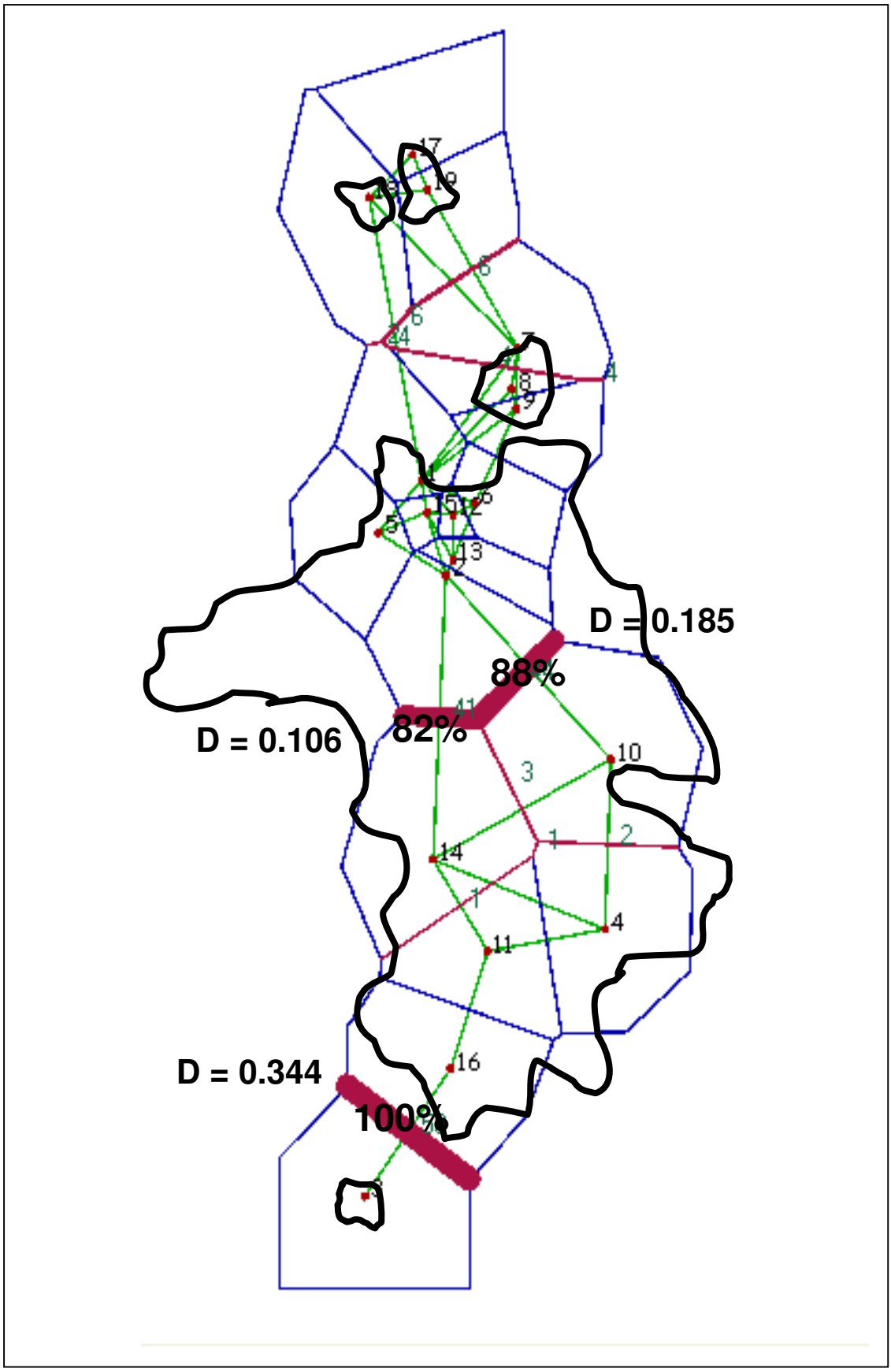

Fig. 41: Strongest barriers to gene flow calculated by the Monmonier's maximum difference algorithm on a set of 50 matrices. The populations (red points) of *Amphitmetus transversus* are plotted in a Delauny triangulation within a Voronoi diagram. The boundaries of the Kakamega Forest are outlined black. The thickness of the barrier (pink lines) indicates the robustness of the barrier during a jack-knife analysis. Distances between adjacent populations are given.

4.1.13. Population Phenogram on NEI's Genetic Distance

The UPMGA analysis on the genetic relationship was based on NEI'S (1978) genetic distance (Table 25). Three main population groups could be inferred (Fig. 42). The population of the southern fragment Kaimosi is well separated from the other populations of the forest (D_{mean}= 0.268). Furthermore, there is a splitting in a northern and a southern population group

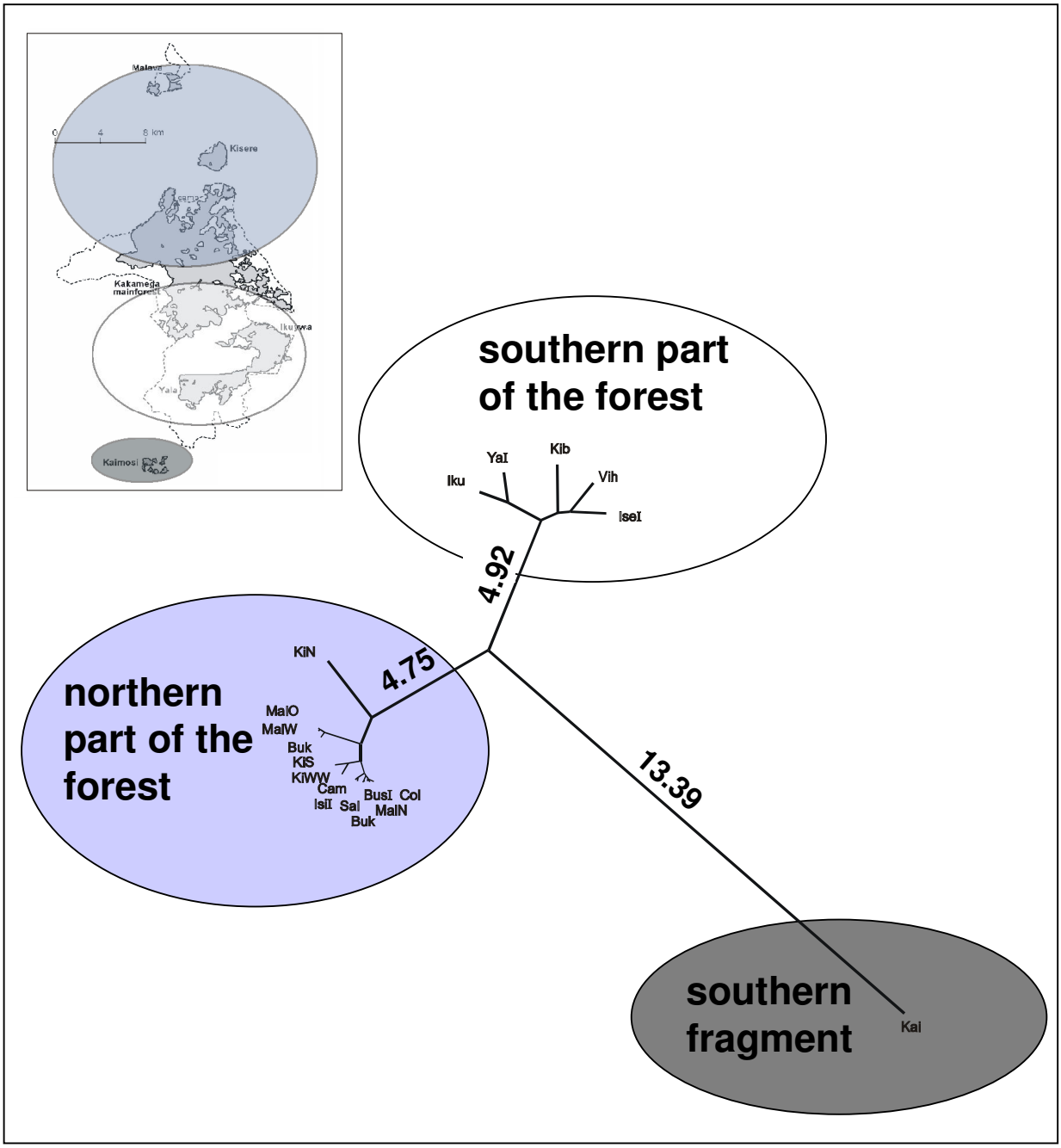

Fig. 42: UPMGA dendrogram of 19 samples of *Amphitmetus transversus* based on NEI's (1978) genetic distance. Estimated branch length between the major clusters is given.

(D_{mean} = 0.145). Distances between the regions are large compared to those between sampling sites within regions (northern part: D_{mean} = 0.023; southern part: D_{mean} = 0.039). Within the southern populations a separation of Ikuywa and Yala I from the three others (D_{mean} = 0.047) could be found. Distances between populations of the northern part were not significant for the most cases (see also pairwise F_{ST} values). The population KiN is most distant to all other populations (D_{mean} = 0.050), while the two other populations located in the moderate fragment – KiS and KiWW – constitute a separated group. Furthermore, two populations of the northern fragment Malawa (MalW; MalO) form a separate group closely related to a population in the northern part of the continuous forest (Buk), while the third Malawa population (MalN) clusters with several populations of the northern part of the continuous forest. Within the regional groups populations are mixing up regardless of geographical distance.

As genetic distances, and hence branch length, are low, the interpretation of within population structure should be treated with caution. The main result of this analysis confirms the result obtained by the Monmonier's maximum difference algorithm. Hence, two main borders to gene flow could be defined. The first separates the southern fragment from the remaining populations, while the second causes a grouping of the northern and the southern populations respectively.

Table 25: NEI's (1978) unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) between pairs of populations in *Amphitmetus transversus.* Site code follows Table 2.

Site	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalN	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO
Col	****	0.997	0.810	0.806	0.979	0.990	0.964	0.987	0.983	0.871	0.776	0.994	0.995	0.933	0.993	0.887	0.998	0.968	0.975
BusI	0.003	****	0.832	0.798	0.966	0.988	0.944	0.979	0.987	0.869	0.774	0.989	0.990	0.922	0.993	0.870	0.999	0.964	0.966
Kai	0.210	0.184	****	0.656	0.784	0.789	0.798	0.800	0.838	0.696	0.638	0.799	0.783	0.706	0.824	0.732	0.798	0.757	0.771
Iku	0.216	0.226	0.421	****	0.818	0.827	0.869	0.817	0.836	0.945	0.979	0.807	0.788	0.948	0.796	0.960	0.800	0.833	0.812
Buk	0.022	0.035	0.244	0.202	****	0.966	0.956	0.977	0.956	0.906	0.815	0.976	0.980	0.941	0.978	0.914	0.979	0.994	0.999
IsiI	0.010	0.013	0.238	0.191	0.035	****	0.960	0.977	0.986	0.882	0.798	0.997	0.986	0.948	0.991	0.895	0.995	0.963	0.967
KiN	0.036	0.058	0.225	0.141	0.045	0.040	****	0.961	0.951	0.884	0.838	0.964	0.938	0.946	0.943	0.955	0.949	0.938	0.946
KiWW	0.014	0.021	0.223	0.202	0.023	0.024	0.040	****	0.991	0.904	0.816	0.982	0.980	0.950	0.978	0.900	0.987	0.964	0.968
KiS	0.018	0.014	0.177	0.179	0.045	0.014	0.051	0.009	****	0.905	0.826	0.983	0.975	0.947	0.981	0.895	0.985	0.949	0.950
Vih	0.138	0.140	0.363	0.057	0.099	0.125	0.123	0.101	0.100	****	0.978	0.869	0.869	0.975	0.868	0.963	0.882	0.923	0.900
YalN	0.253	0.256	0.450	0.021	0.205	0.225	0.177	0.204	0.192	0.023	****	0.777	0.761	0.940	0.769	0.956	0.783	0.837	0.805
Cam	0.006	0.011	0.224	0.214	0.025	0.003	0.037	0.019	0.017	0.141	0.253	****	0.992	0.935	0.993	0.888	0.996	0.969	0.977
Sal	0.005	0.010	0.244	0.238	0.020	0.014	0.064	0.021	0.025	0.140	0.274	0.009	****	0.925	0.998	0.862	0.996	0.974	0.983
IseI	0.070	0.081	0.348	0.053	0.061	0.053	0.056	0.051	0.054	0.026	0.062	0.067	0.078	****	0.926	0.971	0.938	0.948	0.936
BuyI	0.007	0.007	0.194	0.228	0.023	0.009	0.058	0.022	0.019	0.142	0.263	0.007	0.002	0.077	****	0.868	0.997	0.972	0.981
Kib	0.121	0.139	0.312	0.041	0.091	0.111	0.046	0.105	0.111	0.038	0.045	0.118	0.149	0.030	0.142	****	0.881	0.915	0.904
MalN	0.002	0.001	0.225	0.223	0.021	0.005	0.052	0.013	0.015	0.126	0.245	0.004	0.004	0.064	0.003	0.127	****	0.980	0.981
MalW	0.033	0.037	0.279	0.183	0.006	0.037	0.064	0.037	0.052	0.080	0.178	0.031	0.027	0.054	0.028	0.089	0.021	****	0.999
MalO	0.025	0.035	0.260	0.208	0.001	0.034	0.056	0.033	0.052	0.105	0.217	0.024	0.017	0.066	0.020	0.101	0.019	0.001	****

4.2. *Population Genetics of Monolepta vincta*

4.2.1. Genetic Variability of the Microsatellite Markers

Nine microsatellite markers containing dinucleotid repeat motives were developed for the leaf beetle *Monolepta vincta*, which could be collected from seven sample sites (Table 26). The number of individuals collected at each sample site is quite different and ranges from five (MaO) to 41 (IsiII).

Table 26: Allelic diversity in *Monolepta vincta* populations with N = number of sampled individuals, n = number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity. Site code follows Table 2.

	Site	Col	YalII	Cam	BusII	IsiII	IseI	MalO	average
	N	28	22	18	22	41	12	5	
Mv-MS04	n	3	3	3	3	3	3	3	3.00
	H_O	0.29	0.36	0.44	0.36	0.37	0.25	0.40	0.35
	H_E	0.40	0.52	0.57	0.44	0.40	0.36	0.51	0.46
Mv-MS06	n	12	10	11	11	13	9	4	10.00
	H_O	0.54	0.68	0.22	0.36	0.61	0.50	0.40	0.47
	H_E	0.80	0.83	0.83	0.83	0.88	0.87	0.80	0.83
Mv-MS11	n	4	2	3	3	2	2	2	2.57
	H_O	0.11	0.05	0.06	0.05	0.02	0.08	0.00	0.05
	H_E	0.23	0.13	0.25	0.13	0.02	0.08	0.36	0.17
Mv-MS15	n	2	2	2	3	4	2	2	2.43
	H_O	0.04	0.05	0.00	0.00	0.02	0.00	0.00	0.02
	H_E	0.10	0.13	0.11	0.17	0.28	0.16	0.34	0.19
Mv-MS21	n	7	7	7	8	8	8	3	6.86
	H_O	0.78	0.86	0.72	0.77	0.76	0.83	0.60	0.76
	H_E	0.79	0.81	0.81	0.69	0.81	0.83	0.49	0.74
Mv-MS43	n	10	12	9	11	11	4	2	8.43
	H_O	0.32	0.36	0.56	0.64	0.29	0.58	0.00	0.39
	H_E	0.80	0.82	0.83	0.73	0.76	0.77	0.34	0.72
Mv-MS60	n	8	4	7	5	6	5	3	5.43
	H_O	0.14	0.23	0.28	0.18	0.27	0.17	0.20	0.21
	H_E	0.69	0.46	0.72	0.59	0.59	0.58	0.60	0.60
Mv-MS81	n	12	9	10	9	16	6	4	9.43
	H_O	0.50	0.09	0.28	0.36	0.32	0.25	0.00	0.26
	H_E	0.83	0.82	0.87	0.79	0.82	0.78	0.69	0.80
MvMS84	n	4	2	4	3	7	2	2	3.43
	H_O	0.21	0.05	0.22	0.14	0.10	0.33	0.00	0.15
	H_E	0.52	0.49	0.49	0.49	0.59	0.51	0.32	0.49
	n sum	62	51	56	56	70	41	25	
	n mean	6.89	5.67	6.22	6.22	7.78	4.56	2.78	
	H_O mean	0.32	0.30	0.31	0.32	0.31	0.33	0.18	
	H_E mean	0.57	0.55	0.61	0.54	0.57	0.55	0.49	

The total number of alleles per locus and population range from 2 to 16. The mean number of alleles per marker across all populations ranges from 2.43 (Mv-MS15) to 10.00 (Mv-MS06), while the mean number of alleles per population across all loci ranges between 2.78 (MalO) and 7.78 (IsiII). The mean expected heterozygosity per locus and population ranges from 0.02 (Mv-MS11; IsiII) to 0.88 (Mv-MS06; IsiII), while the mean observed heterozygosity ranges from 0.00 to 0.86 (Mv-MS21; YalII). Across all loci a total of 95 different alleles was detected with a maximum of 70 alleles at the population IsiII, which also includes the most individuals. At MalO only 25 alleles were found, which was probably due to its small sample size. A positive relationship was found between sample size and the number of alleles, which followed a linear regression ($R^2 = 0.86$, d.f. = 5, $p < 0.01$; Fig. 43). The smallest population MalO was not considered in the further analyses, because the results are probably biased by the non representative sample size.

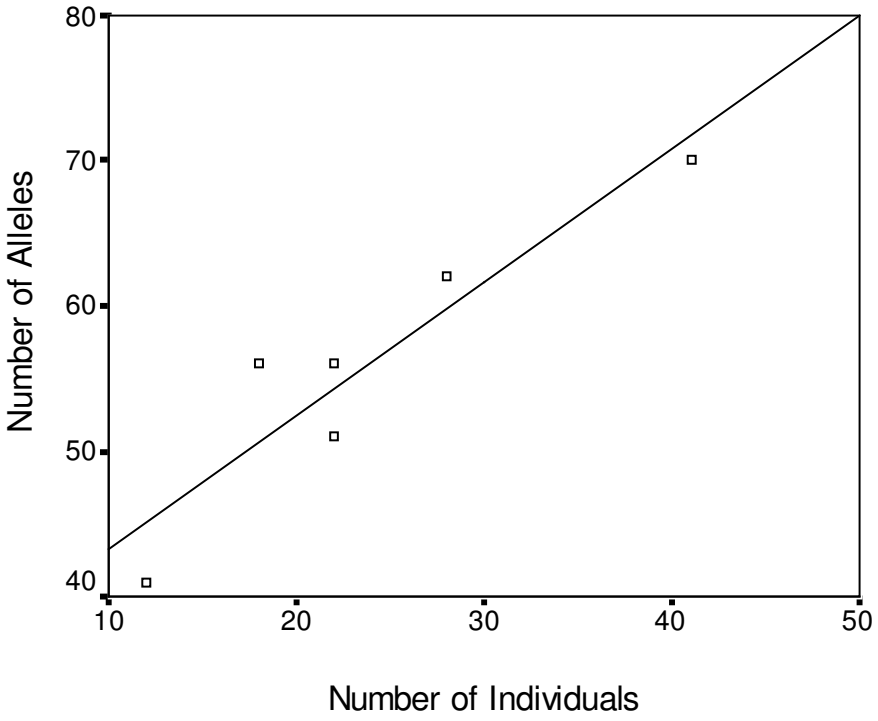

Fig. 43: Relationship between the number of alleles and the number of sampled individuals in *Monolepta vincta*. Number of alleles across all loci show linear correlation ($R^2 = 0.86$, d.f. = 5; $p < 0.01$) to the number of sampled individuals.

4.2.2. Genotypic Linkage Disequilibrium and Hardy-Weinberg Proportion

No genotypic linkage disequilibrium was found between any pair of loci across six populations (Table 27) as well as between any pair of loci for each population. Of 252 pairwise comparisons no significant result appeared even without a sequential Bonferroni correction (RICE 1989). Therefore loci can be treated as independent.

Table 27: Genotypic linkage disequilibrium for each locus pair across six populations in *Monolepta vincta*. NS = not significant

Locus pair			Chi^2	d.f.	significance
MS04	&	MS06	14.96	12	NS
MS04	&	MS11	7.66	12	NS
MS06	&	MS11	9.23	12	NS
MS04	&	MS15	11.03	12	NS
MS06	&	MS15	7.54	12	NS
MS11	&	MS15	0.00	12	NS
MS04	&	MS21	1.57	12	NS
MS06	&	MS21	2.89	12	NS
MS11	&	MS21	10.55	12	NS
MS15	&	MS21	7.97	12	NS
MS04	&	MS60	14.41	12	NS
MS06	&	MS60	5.53	12	NS
MS11	&	MS60	4.23	8	NS
MS15	&	MS60	10.76	12	NS
MS21	&	MS60	13.08	12	NS
MS04	&	MS81	16.36	12	NS
MS06	&	MS81	7.04	12	NS
MS11	&	MS81	2.31	12	NS
MS15	&	MS81	11.91	12	NS
MS21	&	MS81	3.90	12	NS
MS60	&	MS81	12.08	12	NS
MS04	&	MS84	15.20	12	NS
MS06	&	MS84	11.09	12	NS
MS11	&	MS84	5.57	10	NS
MS15	&	MS84	10.64	12	NS
MS21	&	MS84	2.78	12	NS
MS60	&	MS84	7.85	12	NS
MS81	&	MS84	13.73	12	NS
MS04	&	MS43	10.04	12	NS
MS06	&	MS43	7.01	12	NS
MS11	&	MS43	6.38	12	NS
MS15	&	MS43	16.23	12	NS
MS21	&	MS43	9.24	12	NS
MS60	&	MS43	4.10	12	NS
MS81	&	MS43	6.87	12	NS
MS84	&	MS43	13.23	12	NS

A significant deviation from Hardy-Weinberg proportion was observed at 8 of 9 loci (Table 28) after sequential Bonferroni correction. Deviations were all heterozygote deficiencies. One marker (Mv-MS21) is in equilibrium, which indicates that the deficiencies were probably caused by non amplifying alleles, because inbreeding or WAHLUND-Effects are expected to yield a deficit over all loci (MORAND *et al*. 2002, VAN OOSTERHOUT *et al*. 2004). Similar to the results of *Amphitmetus transversus* it was suspected that null alleles cause the observed pattern. The significant differences between the observed and expected proportion of heterozygotes is also reflected in each of the seven populations over all loci (Fig. 44).

Table 28: Hardy-Weinberg exact tests for each locus across six populations in *Monolepta vincta*

Locus	Chi^2	d.f.	P-value
Mv-MS04	24.4	12	0.02*
Mv-MS06	> 100	12	0.00***
Mv-MS11	34.2	8	0.00***
Mv-MS15	77.4	12	0.00***
Mv-MS21	12.7	12	0.39
Mv-MS43	> 100	12	0.00***
Mv-MS60	> 100	12	0.00***
Mv-MS81	> 100	12	0.00***
Mv-MS84	> 100	12	0.00***

*** significance at P<0.001 ** significance at P<0.01

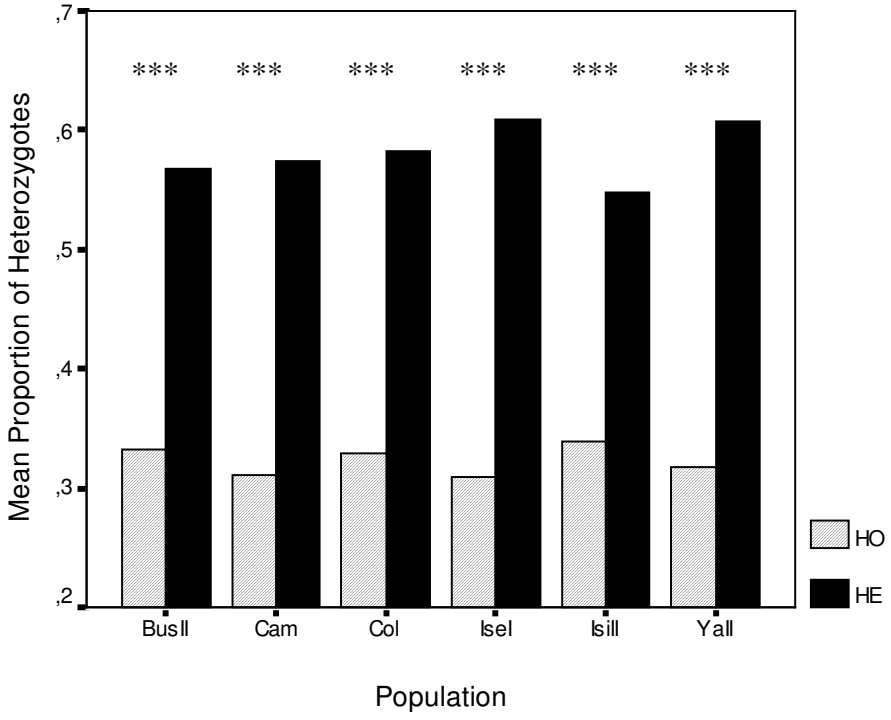

Fig. 44: Expected (HE) and observed (HO) heterozygosity in seven populations of *Monolepta vincta*. The significance of the Hardy-Weinberg exact tests for each population across all loci is given. *** Significance at P < 0.001.

If null alleles caused the observed pattern it is probable to find null-homozygotes in the genotype matrix of the concerned markers. Regarding the matrix of genotypes several individuals did not yield an amplification product at particular markers (Appendix, Table A3). Six of nine loci show the expected pattern, which may indicate null-homozygotes (Table 29). Also Mv-MS21 lacks of some amplification products, although the marker is in Hardy-Weinberg-Equilibrium and null alleles are not expected. Furthermore, null-homozygotes were not detected in Mv-MS04, Mv-MS06 and Mv-MS11. Mv-MS06 is the most variable locus of the set. Hence, homozygote individuals are extremely rare, which might be a reason for the missing null-homozygote. The marker Mv-MS04 is not in Hardy-Weinberg-Equilibrium, although the deviation is much weaker than for the seven other loci. Perhaps, null-alleles at this marker are present in low frequency and therefore null-homozygotes do not show up. However, there is no reasonable explanation for the missing null-homozygote at At-MS11.

The observable pattern of the non-amplifying genotypes does not fit completely the expected pattern of null-homozygotes. Nevertheless, the conformity to Hardy-Weinberg expectations in Mv-MS21 is an important indication for the existence of null alleles at the remaining markers. Hence, the observed distribution of missing genotypes might be caused by other genotyping errors (BONIN *et al.* 2004).

Table 29: Number of non amplifying genotypes (n) per marker in *M. vincta*.

Loci	n
Mv-MS04	-
Mv-MS06	-
Mv-MS11	-
Mv-MS15	2
Mv-MS21	3
Mv-MS43	4
Mv-MS60	22
Mv-MS81	4
Mv-MS84	7

4.2.3. Evidence of Non-Amplifying Alleles

The probability of null alleles causing the deviations from Hardy-Weinberg expectations was also tested in the program MICRO-CHECKER (VAN OOSTERHOUT *et al*. 2004). Evidence for null alleles was found in several populations at seven of nine loci (Table 30). As already expected Mv-MS21 did not show a probability of containing null-alleles. Furthermore, there was no evidence of null alleles found in Mv-MS04. This marker showed a significant deviation from Hardy-Weinberg expectation after sequential Bonferroni correction (RICE 1989), although the level of significance was lower than for the other seven markers. The frequencies of the null alleles of the seven markers were statistically corrected under the assumption that each population is in Hardy-Weinberg-Equilibrium (CHAKRABORTY *et al* 1992, BROOKFIELD 1996) using the estimation "Brookfield 1" (BROOKFIELD 1996). The estimate does not account for individuals that did not yield any amplification product. It was

Table 30: Evidence of null alleles estimated with the program MICRO-CHECKER for populations of *Monolepta vincta*. Ho_E = total number of expected homozygotes, Ho_O = total number of observed homozygotes, NA = null alleles.

	Mv-MS04			Mv-MS06			Mv-MS11			Mv-MS15			Mv-MS21		
Site	NA	Ho_O	Ho_E	NA	Ho_O	Ho_E	NA	Ho_O	Ho_E	NA	Ho_O	Ho_E	NA	Ho_O	Ho_E
Col		20.00	16.91	x	13.00	6.09	x	25.00	21.68		27.00	25.16		6.00	6.50
YalII		14.00	10.84		7.00	4.20		21.00	19.20		21.00	19.20		3.00	4.66
Cam		10.00	8.11	x	14.00	3.47	x	17.00	13.58		18.00	16.11		5.00	3.92
BusII		14.00	12.42	x	14.00	4.16		21.00	19.16	x	22.00	18.27		3.00	5.30
IsiII		26.00	24.67		16.00	5.22		40.00	40.01		39.00	28.61		10.00	8.39
Ise1		9.00	7.88		6.00	1.96		11.00	11.04		12.00	10.16		2.00	2.42

	Mv-MS43			Mv-MS60			Mv-MS81			Mv-MS84		
	NA	Ho_O	Ho_E	NA	Ho_O	Ho_E	NA	Ho_O	Ho_E	NA	Ho_O	Ho_E
Col	x	19.00	6.02	x	22.00	7.19	x	13.00	4.11	x	22.00	13.59
YalII	x	14.00	4.32	x	12.00	7.21	x	19.00	3.48	x	21.00	11.57
Cam	x	8.00	3.53	x	13.00	5.33	x	13.00	2.69	x	14.00	9.39
BusII		8.00	6.23	x	14.00	5.33	x	13.00	4.07	x	19.00	11.48
IsiII		26.00	7.16		23.00	10.16		26.00	5.90		32.00	12.06
Ise1		5.00	3.13		7.00	2.44		9.00	2.71		8.00	6.17

x null alleles may be present at this locus, as is suggested by the generall excess of homozygotes for the most allele size classes

chosen, since the pattern of non amplifying alleles is probably not only caused by null alleles, but biased by other genotyping errors. The estimated frequency of the null alleles was rather high. In several populations the null alleles had an estimated frequency of 30 % and more (Appendix Table A17–A25). The lowest frequency is found for Mv-MS11, which might be a reason for the missing null-homozygotes at this marker. Null homozygotes are rare. Even at estimated frequencies of 10 % only 1 of 100 individuals is expected to be a null homozygote (VAN OOSTERHOUT *et al.* 2004).

MICRO-CHECKER (VAN OOSTERHOUT *et al.* 2004) allowed an adjustment of genotypes according to null allele frequencies. These genotypes were used to generate a new matrix (Appendix Table A4). Based on the adjusted matrix several tests were re-analysed and compared to the results of the original data set.

4.2.4. Allele Frequency Distribution

The number of alleles per marker is quite different and ranges between three (Mv-MS04) and 20 (Mv-MS81) (Appendix Fig A2). The markers Mv-MS11 and Mv-MS15 show one common allele and several others at low proportion (< 0.1), while the most other show at least two common alleles at higher frequencies. The presented loci of *Monolepta vincta* reveal a stepwise increase in length that does not completely fit to an underlying increase in repeat units of the dinucleotid repeat motive. There are several 'gaps', were an increase/decrease of more than two basepairs occur. The frequencies of alleles at Mv-MS21 show distribution with an allele of the highest frequency at an intermediate allele size class and a continuous increase or decrease in allele frequency of smaller and larger alleles. Such a clear pattern is not represented in the remaining markers and also unusual following reports for microsatellites (LINDENMAYER & PEAKALL 2000, GARZA & WILLIAMSON 2001).

No locus appeared to be monomorphic in analysis of the geographic variation of allele frequencies (Fig. 45–53, Appendix Table A17–A25). The distribution reflects the high allelic variability of several markers. Private alleles occur at eight loci except of Mv-MS04 (Table 31). The sample site IsiII contains the highest number of private alleles, which is probably due to its high sample size. However, no private allele appears at IseI, which shows the smallest sample size of the considered populations. The strong dependence of the number of alleles against the sample size is obvious. Nevertheless, the sample site Cam contains a comparable small sample size but five private alleles, which is considerable high. The influence of the sample size on the number of detected alleles is also reflected in the general allelic variability

of the most markers. A high proportion of null alleles at several markers was found. It is likely that more that one non amplifying allele is responsible for the observed pattern. At least there is no remarkable geographically structuring, which is consistent across the markers, although several rare alleles are clustered into regionally groups. For example the allele '12' of Mv-MS60 was only represented in three populations of the north eastern part of the forest (IsiII, BusII and Cam), and allele '12' of Mv-MS11 could only be found in three of the northern populations (Col, BusII and Cam). The null allele of Mv-MS15 on the other hand occured only in populations located close to the river Isiukhu, namely IsiII and BusII. Nevertheless the trends are not convincing as rare alleles are also shared between populations of larger geographical scale, for example the allele '13' at Mv-MS11, that is included in the southern population IseI as well as in more distant population IsiII and BusII.

Table 31: Private alleles of nine microsatellite markers in six populations of *Monolepta vincta*. N = number of sampled individuals. ID of private alleles is given, which follows Table A11–A25 in the Appendix).

	BusII	Col	Cam	YalII	IsiII	IseI
N	22	28	18	22	41	12
Mv-MS04						
Mv-MS06				24		
Mv-MS11		15			16	
Mv-MS15	13				14, 15	
Mv-MS21	20					
Mv-MS43		28	13, 14	16, 22	26	
Mv-MS60		20	19			
Mv-MS81		11	12, 13		24, 29	
Mv-MS84					15	

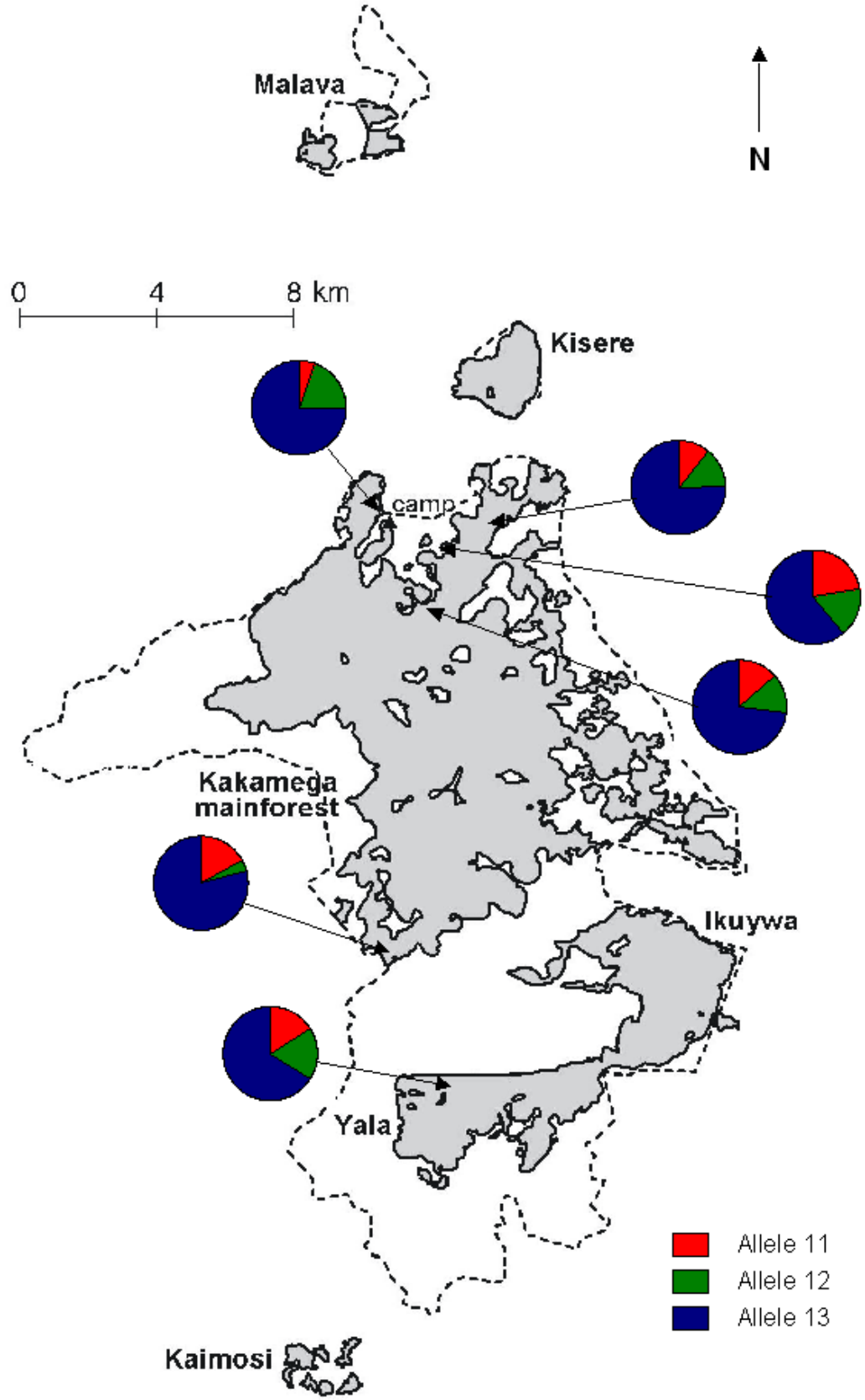

Fig. 45: Geographic variation of allele frequencies in the marker Mv-MS04 across the six populations of *Monolepta vincta*.

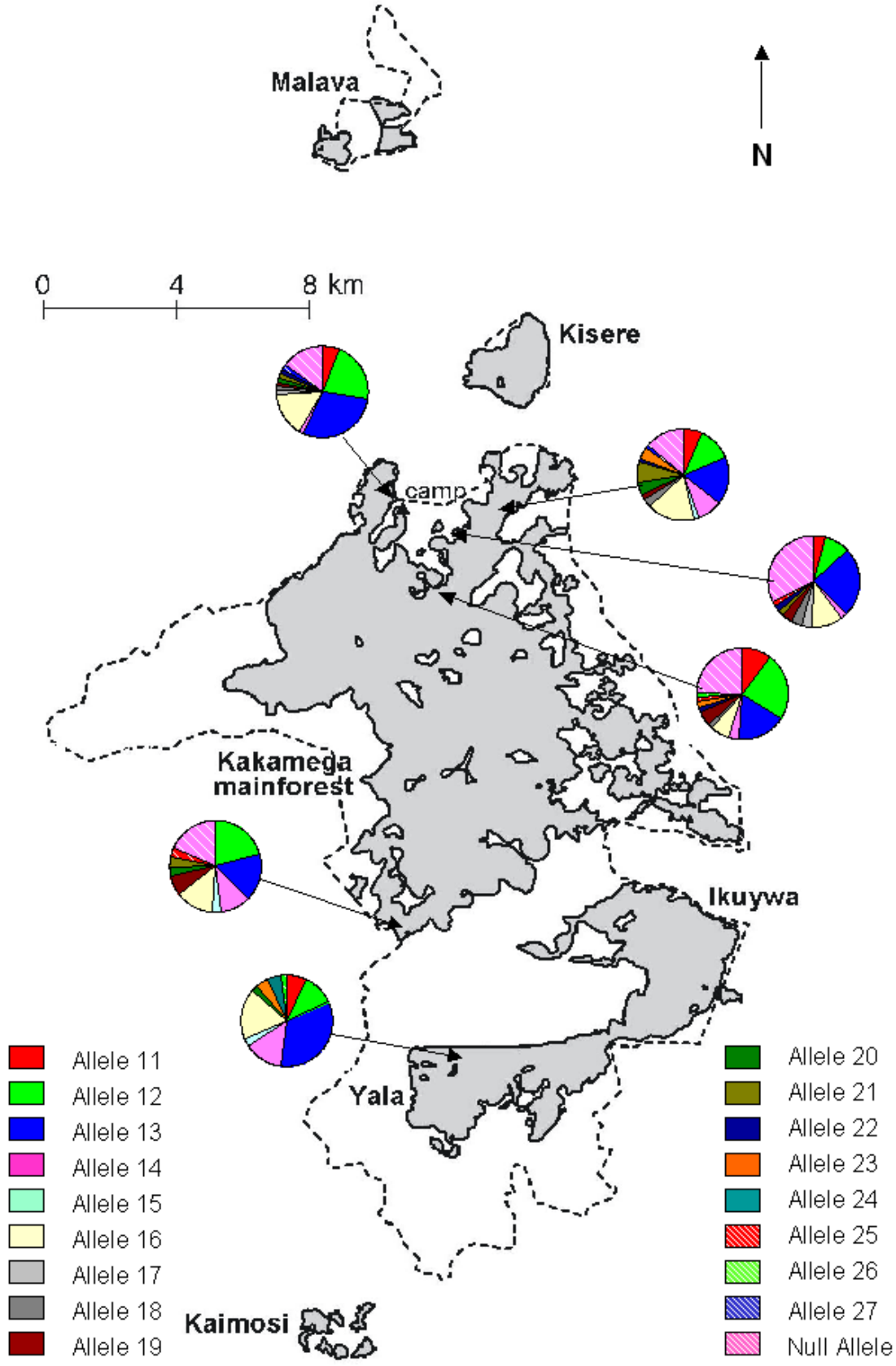

Fig. 46: Geographic variation of allele frequencies in the marker Mv-MS06 across the six populations of *Monolepta vincta*.

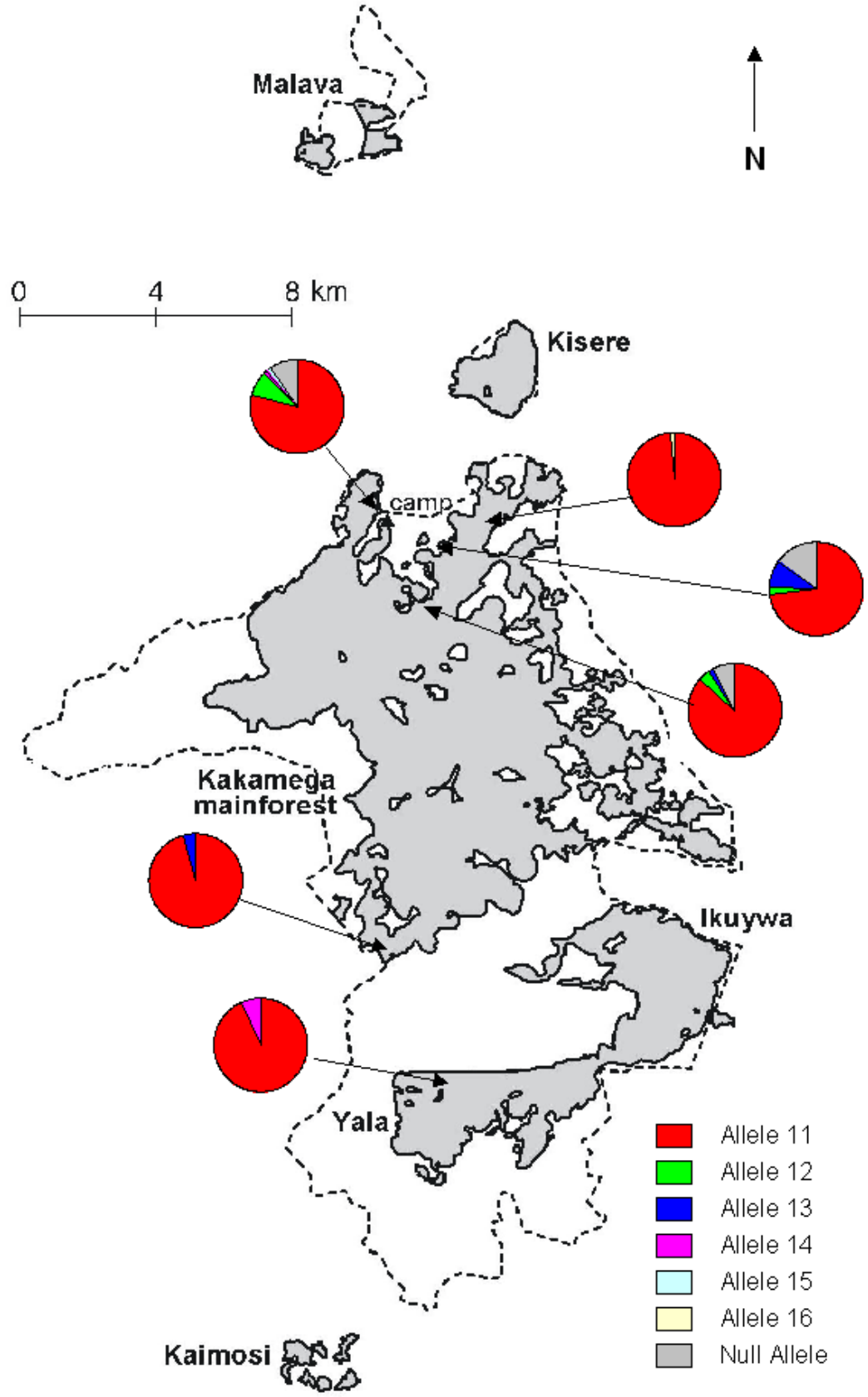

Fig. 47: Geographic variation of allele frequencies in the marker Mv-MS11 across the six populations of *Monolepta vincta*.

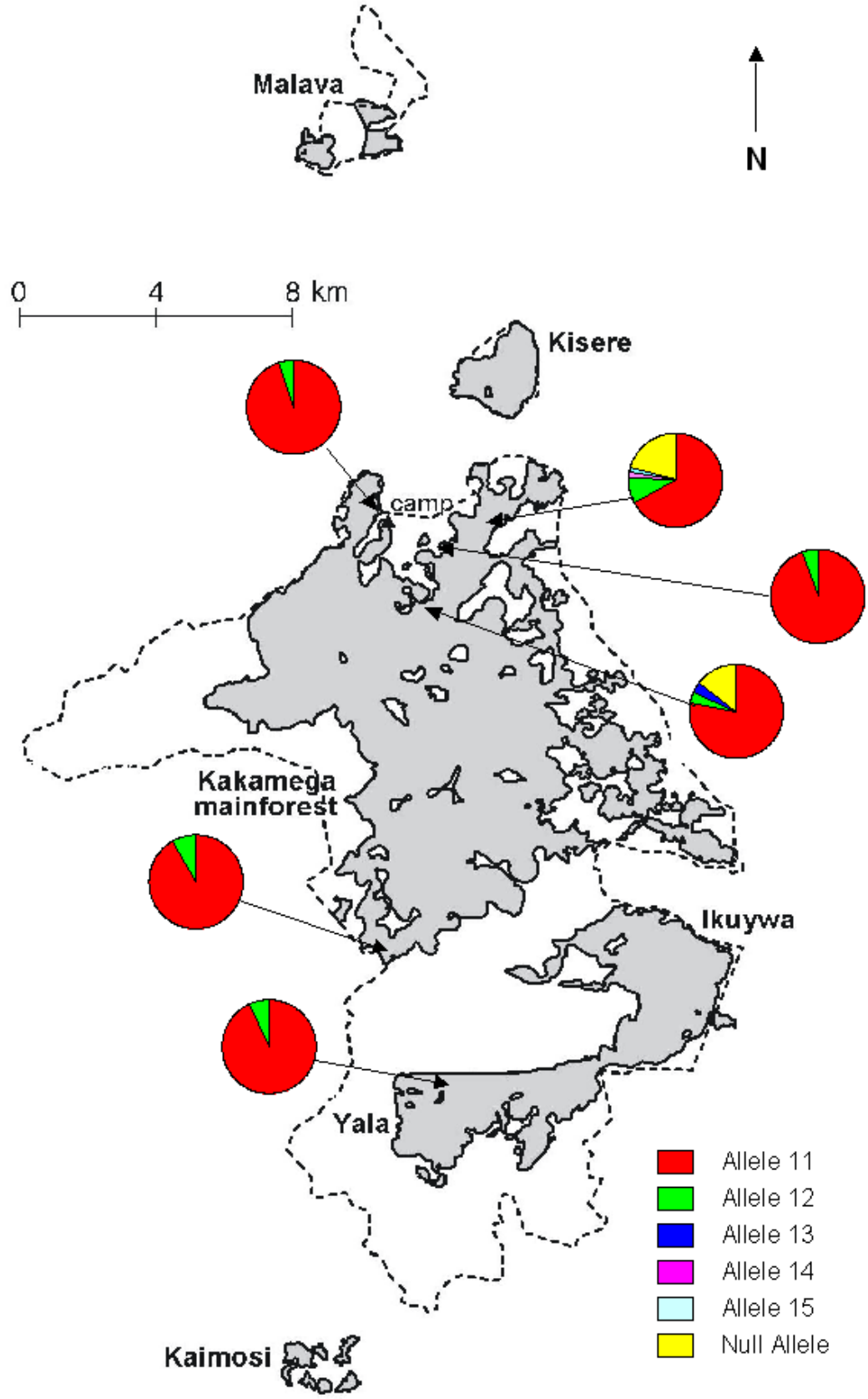

Fig. 48: Geographic variation of allele frequencies in the marker Mv-MS15 across the six populations of *Monolepta vincta*.

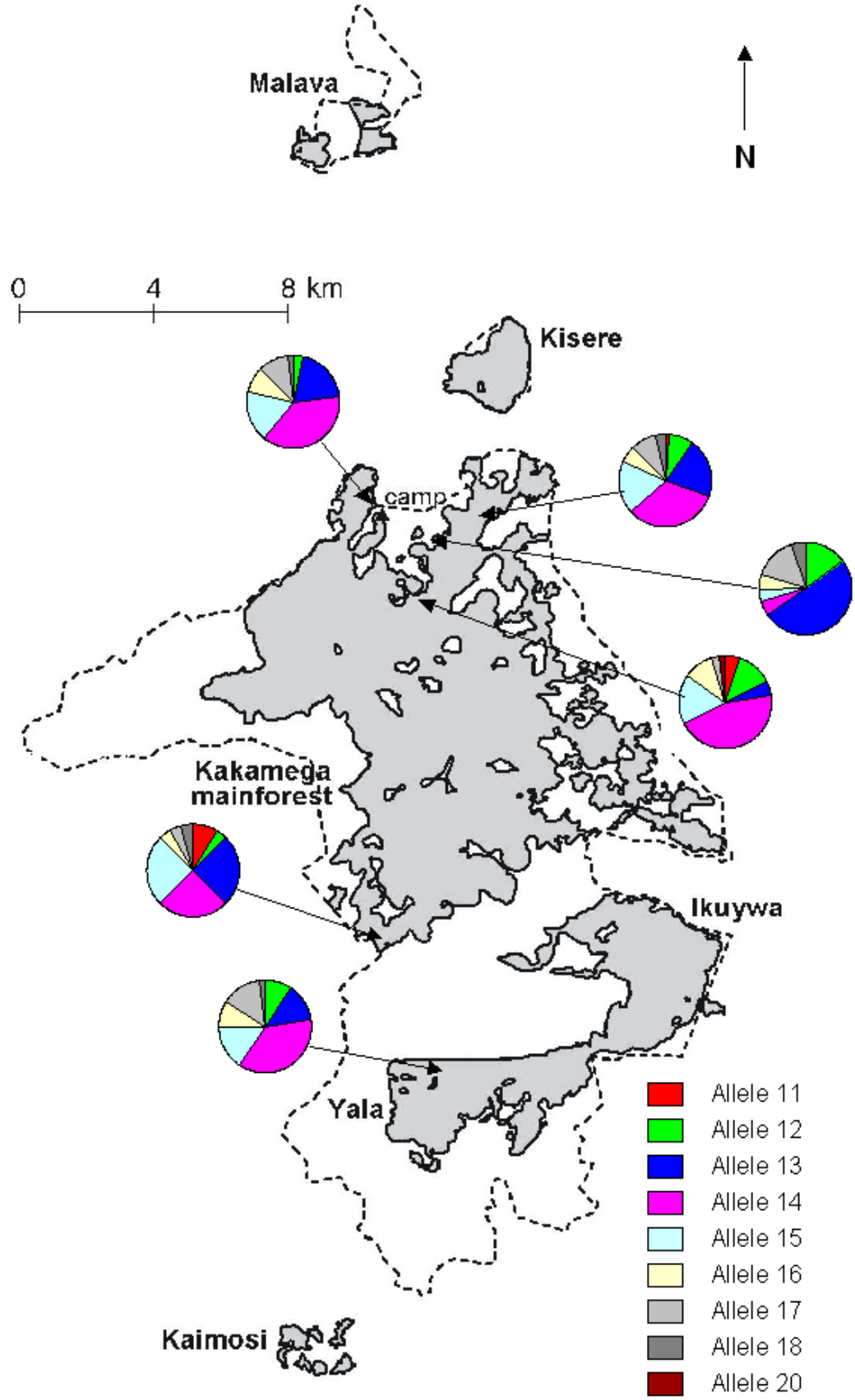

Fig. 49: Geographic variation of allele frequencies in the marker Mv-MS21 across the six populations of *Monolepta vincta*.

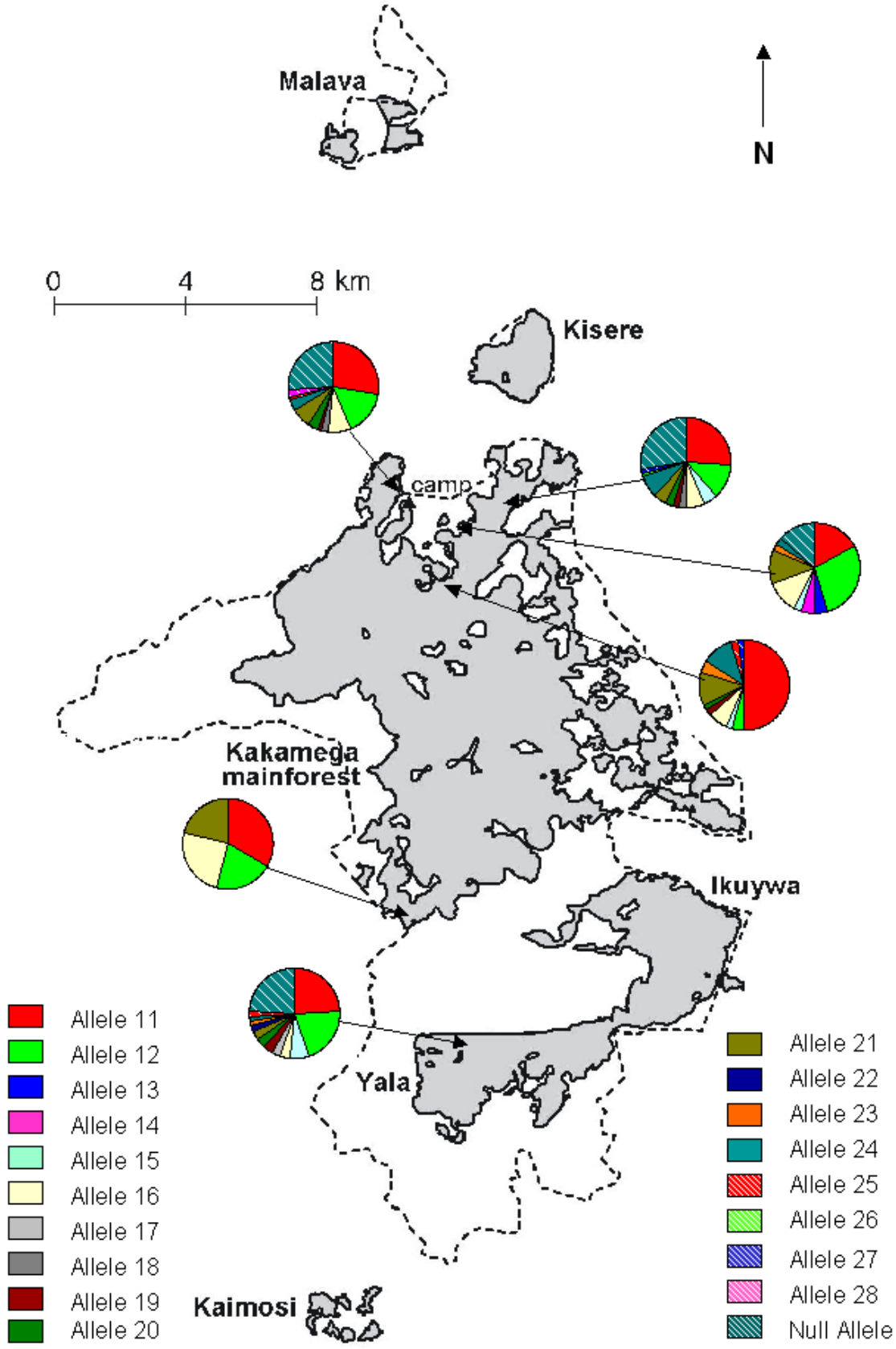

Fig. 50: Geographic variation of allele frequencies in the marker Mv-MS43 across the six populations of *Monolepta vincta*.

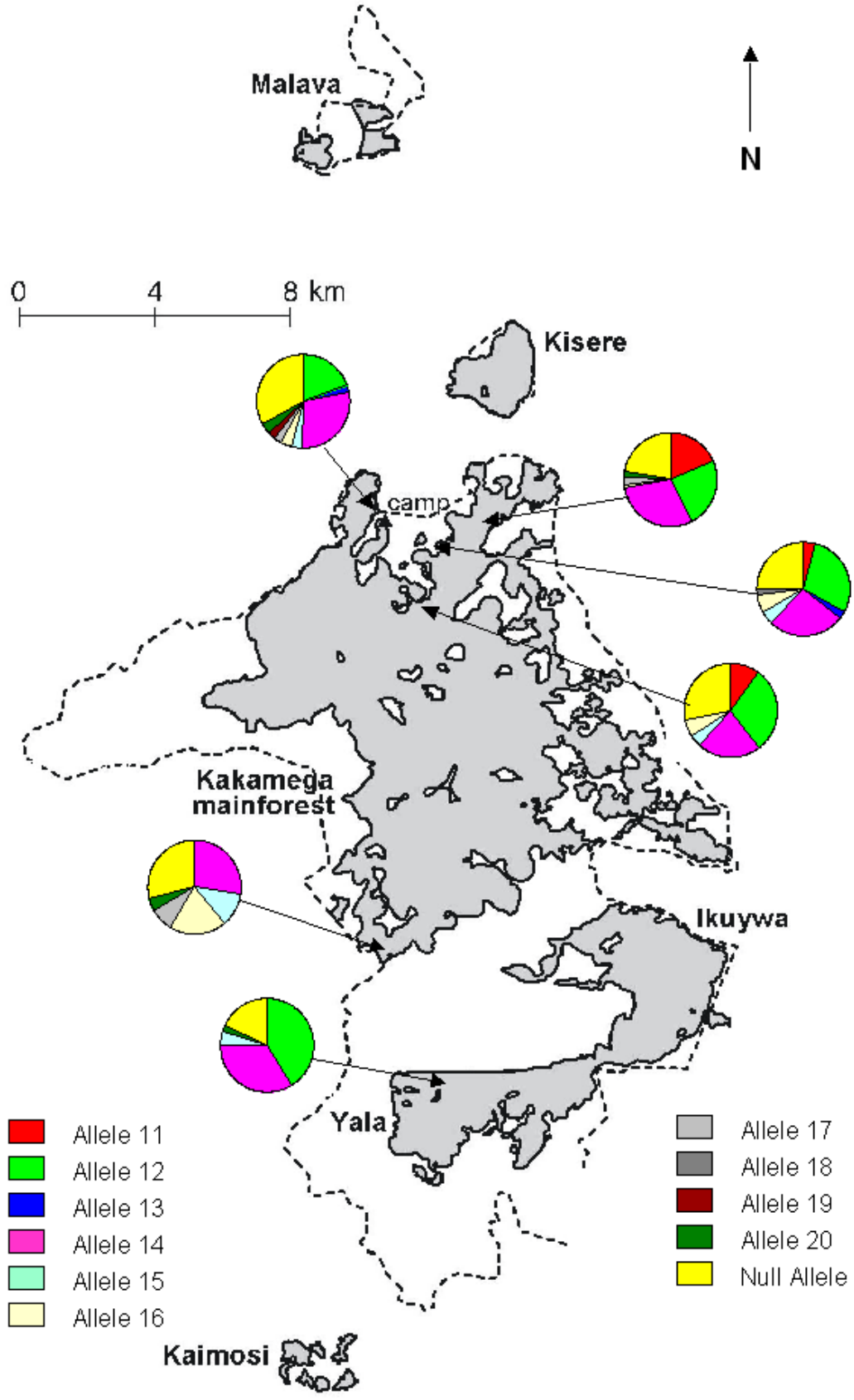

Fig. 51: Geographic variation of allele frequencies in the marker Mv-MS60 across the six populations of *Monolepta vincta*.

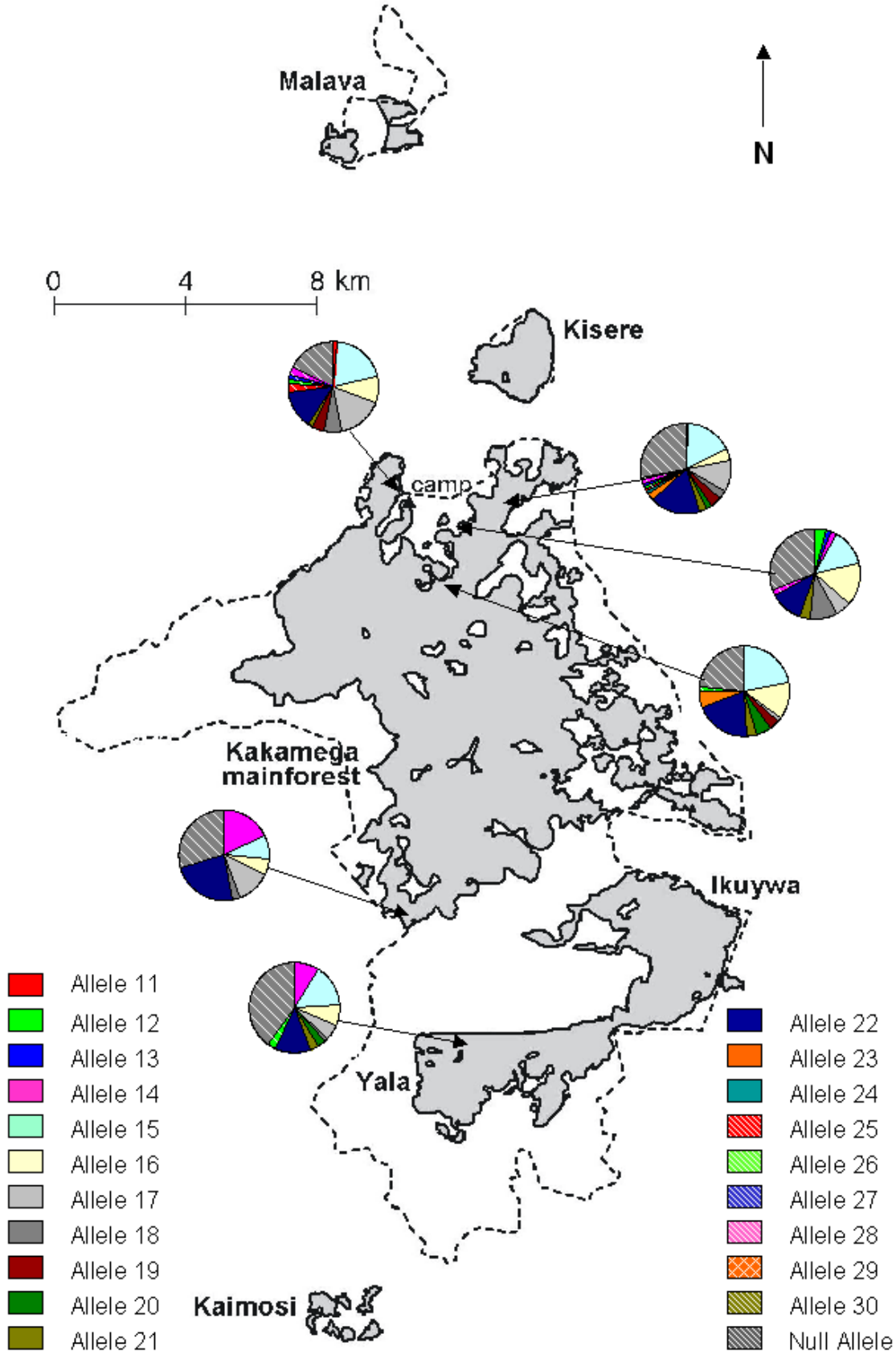

Fig. 52: Geographic variation of allele frequencies in the marker Mv-MS81 across the six populations of *Monolepta vincta*.

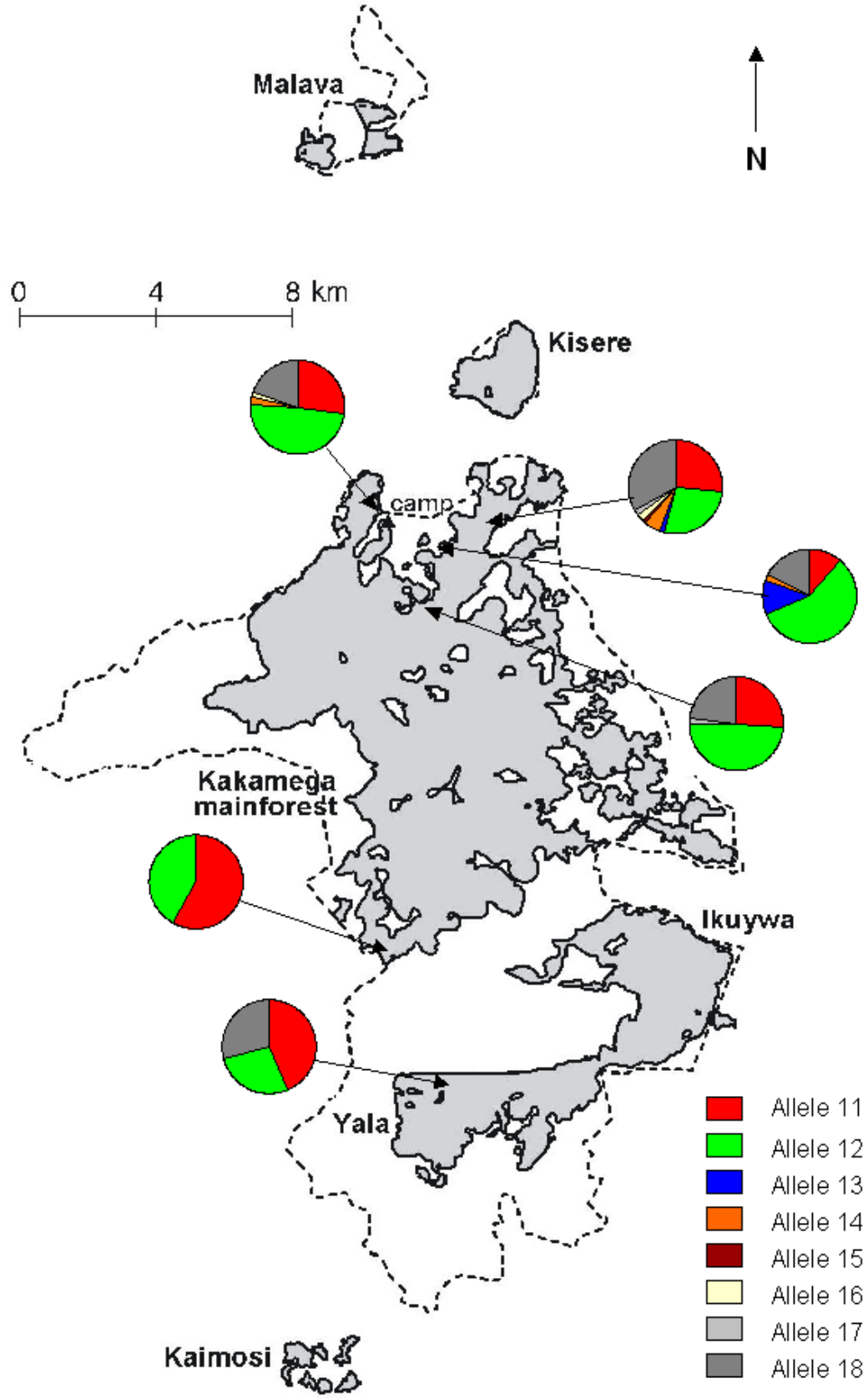

Fig. 53: Geographic variation of allele frequencies in the marker Mv-MS84 across the six populations of *Monolepta vincta*.

4.2.5. Test on Genetic Differentiation

The values of pairwise F_{ST} range between –0.004 (Col – Cam) and 0.024 (IseI – BusII) (Table 32). Nine of 15 pairwise distances were significant after a sequential Bonferroni correction (RICE 1989) for multiple comparisons. The distribution of significant differences showed no general pattern, which could be explained by geographical distances. All comparisons including Col were not significant except of one between Col and IsiII. In general the pairwise genetic differences are rather low with a maximum of 2.43 % variation found between a pair of population. Genic differentiation across all populations was highly significant for each locus and over all loci (Chi^2 = infinity, d.f. = 18, $P < 0.001$). However, genic differentiation was not significant at four loci (Mv-MS04: P = 0.36, Mv-MS06: P = 0.10, Mv-MS15: P = 0.67 and Mv-MS21: P = 0.58).

Table 32: Pairwise F_{ST} estimates of *M. vincta* across all loci on the upper matrix. The lower matrix indicates the significance for pairwise comparisons after sequential Bonferroni correction: NS indicates no significant differences; ** significance at $P < 0.01$; ***significance at $P < 0.001$. Site code follows Table 2.

Site	BusII	Col	Cam	YalII	IsiII	IseI
BusII		–0.0031	0.0147	0.0112	0.0030	0.0243
Col	NS		–0.0038	0.0016	–0.0004	0.0065
Cam	***	NS		0.0163	0.0150	0.0230
YalII	**	NS	***		–0.0007	0.0081
IsiII	NS	**	***	NS		0.0031
IseI	***	NS	**	***	***	

The outcome of the F-statistics confirmed the result of the low genetic differentiation found between pairs of populations. The value for F_{ST} (0.006) indicates a very low genetic differentiation among the six populations of *Monolepta vincta* (Table 33). The F_{IS} value over all populations is rather high, indicating a heterozygote deficiency found within single sample sites (see also global statistics of *Monolepta vincta*). High F_{IS} values are found at all markers except of Mv-MS21. It is assumed that the high F_{IS} value is caused by the presence of null alleles, for which evidence was found in seven of the nine markers using the program MICRO-CHECKER (VAN OOSTERHOUT *et al.* 2004).

Table 33: F-statistics of *M. vincta* according to WEIR & COCKERHAM (1984). Estimates were calculated by jack-knifing over loci (SE in brackets). Confidence interval (CI) was computed by bootstrapping (15000 times) over loci.

Locus	f ($F_{wc(IS)}$)	F ($F_{wc(IT)}$)	θ ($F_{wc(ST)}$)
Mv-MS04	0.211 (+/– 0.047)	0.207 (+/– 0.048)	–0.006 (+/– 0.007)
Mv-MS06	0.391 (+/– 0.071)	0.392 (+/– 0.073)	–0.001 (+/– 0.007)
Mv-MS11	0.586 (+/– 0.078)	0.593 (+/– 0.075)	0.018 (+/– 0.023)
Mv-MS15	0.892 (+/– 0.051)	0.891 (+/– 0.053)	–0.010 (+/– 0.021)
Mv-MS21	0.005 (+/– 0.035)	0.002 (+/– 0.031)	–0.004 (+/– 0.009)
Mv-MS43	0.481 (+/– 0.086)	0.481 (+/– 0.077)	0.002 (+/– 0.016)
Mv-MS60	0.646 (+/– 0.055)	0.650 (+/– 0.052)	0.014 (+/– 0.022)
Mv-MS81	0.619 (+/– 0.068)	0.617 (+/– 0.068)	–0.006 (+/– 0.006)
Mv-MS84	0.724 (+/– 0.075)	0.735 (+/– 0.069)	0.040 (+/– 0.034)
All	0.456 (+/– 0.094)	0.459 (+/– 0.095)	0.006 (+/– 0.006)
95 % CI	0.280–0.616	0.280–0.621	–0.003–0.018

The extent of genetic differentiation turns out to be different under consideration of the re-estimates null alleles. As shown in the matrix of pairwise genetic differences (Table 34), 13 of 15 comparisons are significant after sequential Bonferroni correction. The genic differentiation across all populations was highly significant over all loci (Chi^2 = infinity, d.f. = 18, $P < 0.001$). Nevertheless, the genetic differentiation was not significant for single markers. The loci Mv-MS04 ($P = 0.32$), Mv-MS06 ($P = 0.12$), Mv-MS21 ($P = 0.55$) and Mv-MS81 ($P = 0.20$) did not show significance in genic differentiation after sequential Bonferroni correction.

Table 34: Pairwise F_{ST} estimates of *M. vincta* including null alleles across all loci on the upper matrix. The lower matrix indicates the significance for pairwise comparisons after sequencial Bonferroni correction: NS indicates no significant differences; **P < 0.01; ***P < 0.001. Site code follows Table 2

Site	BusII	Col	Cam	YalII	IsiII	IseI
BusII		0.0055	0.0150	0.0191	0.0119	0.0214
Col	***		0.0004	0.0091	0.0132	0.0131
Cam	***	NS		0.0206	0.0289	0.0135
YalII	***	***	***		0.0105	0.0200
IsiII	***	***	***	**		0.0288
IseI	***	**	NS	***	***	

The F-statistics based on the matrix containing null-alleles revealed an estimation of genetic differentiation across populations that is three times as much as the F_{ST} of the original data set (Table 35). The genetic variance across the populations is mainly caused by four markers (Mv-MS11, Mv-MS15, Mv-MS43 and Mv-MS42). These are markers with a high proportion

of estimated null-alleles. The two markers Mv-MS04 and Mv-MS21, which did not show evidence for null alleles, do not indicate any differentiation between populations at all.

Table 35: F-statistics of *M. vincta* according to WEIR & COCKERHAM (1984) based on re-estimated null allele matrix of six populations. Estimates were calculated by jack-knifing over loci (SE in brackets). Confidence interval (CI) was computed by bootstrapping (15000 times) over loci.

	f ($F_{wc(IS)}$)		F ($F_{wc(IT)}$)		θ ($F_{wc(ST)}$)	
Mv-MS04	0.187	(+/– 0.047)	0.183	(+/– 0.048)	–0.005	(+/– 0.007)
Mv-MS06	0.196	(+/– 0.037)	0.199	(+/– 0.039)	0.003	(+/– 0.006)
Mv-MS11	0.289	(+/– 0.078)	0.328	(+/– 0.080)	0.055	(+/– 0.040)
Mv-MS15	0.278	(+/– 0.188)	0.338	(+/– 0.157)	0.097	(+/– 0.051)
Mv-MS21	0.005	(+/– 0.035)	0.002	(+/– 0.031)	–0.004	(+/– 0.009)
Mv-MS43	0.186	(+/– 0.026)	0.200	(+/– 0.026)	0.017	(+/– 0.017)
Mv-MS60	0.151	(+/– 0.048)	0.146	(+/– 0.052)	0.005	(+/– 0.013)
Mv-MS81	0.171	(+/– 0.018)	0.169	(+/– 0.014)	–0.002	(+/– 0.005)
Mv-MS84	0.112	(+/– 0.041)	0.151	(+/– 0.054)	0.043	(+/– 0.022)
All	0.160	(+/– 0.028)	0.172	(+/– 0.030)	0.014	(+/– 0.007)
95 % CI	0.108–0.213		0.117–0.231		0.003–0.030	

The comparison of the two statistics shows that the result might be biased by null alleles. It can not be excluded that both, the original as well as the re-estimated matrix contain incomplete or false information about the genetic structure of the beetle. According to the given results the genetic differentiation between populations of *Monolepta vincta* is extremely low. Furthermore, the existence of null-alleles biases the outcome of the analysis in an unknown extent. Consequently it was decided to omit further analyses on genetic differentiation in *M. vincta*.

Alles Wissen und alle Vermehrung unseres Wissens endet nicht mit einem Schlusspunkt, sondern mit einem Fragezeichen. Ein Plus an Wissen bedeutet ein Plus an Fragestellungen, und jede von ihnen wird immer wieder von neuen Fragestellungen abgelöst.

Hermann Hesse

5. Discussion

5.1. Methods

5.1.1. Sampling

The sampling of an acceptable number of individuals per species turned out to be rather difficult. The majority of the present populations and individual were caught by beating, while canopy fogging yield a large diversity of arthropods but specimens of the beetles of interest were poorly represented in the samples. Hand-sampling methods are superior in sampling populations of one species to tree fogging methods. This does not only refer to the quantity of the collection but also the DNA quality of preserved individuals. DNA of the samples collected by canopy fogging often was degraded, because probes could not be preserved in alcohol immediately after sampling. As a consequence most of the fogging sites were excluded from the analyses, due to large drop-outs in PCR-Amplification caused by DNA degradation.

Although the Kakamega Forest is of limited and manageable size, the access to the central parts of the continuous forest was difficult. Not only missing pathways made the sampling in the centre difficult, but so the height of the canopy, which was not accessible by beating. The difficulties are reflected for example in the missing sampling success at the "Centre" (Cen), where only one specimen could be found. Finally, beating areas were chosen by their accessibility through pathways and the ability to reach smaller canopies and shrubs.

The sampling was restricted to the continuous part of the Kakamega Forest as well as the adjacent fragments Kaimosi, Malawa, Kisere and Bunyala. No specimens were found in the most disturbed Bunyala Fragment, which could not be taken into consideration in the later analyses. The Kakamega Forest represents the most eastern range of the guineo-congolian rainforest block. It was decided to exclude the North Nandi and the South Nandi Forest from sampling, because they are located at an altitude between 1700 and 2130 m and exhibit montane flora and fauna elements, for example the typical montane species *Cyathea*

mannianna. The higher amount of montane species distinguishes the structure of these forests characteristically from that of the Kakamega Forest and the sampling was confined to the area of the latter. The restriction to the forest complex enabled an extensive sampling of *A. transversus* as a large number of populations containing a representative number of individuals were available. The sampling of *M. vincta* turned out to be much more difficult, because the species was not found in every investigated part of the forest. Most of the sampled individuals of *M. vincta* were collected at different sites than *A. transversus*. *M. vincta* was found more often in the area of the fogging sites (BusII, YalII, IsiII). Although the fogging took place at those sites *M. vincta* was mainly caught by hand-sampling. The mentioned areas are characterized by higher canopies and a thicker forest.

5.1.2. Molecular Analyses

5.1.2.1. Variability of Microsatellite Markers

Microsatellites turned out to be highly polymorphic markers and were useful in analysing the population structure of the beetles on the small spatial scale of the present study. Earlier tests on polymorphism in mitochondrial sequences (PATT, unpublished) did not yield any variability, whereas microsatellites contained useful information on the population genetic level. The variability of the established marker system in *A. transversus* and *M. vincta* shows considerable differences (Fig. 54). Most of the six markers of *A. transversus* are

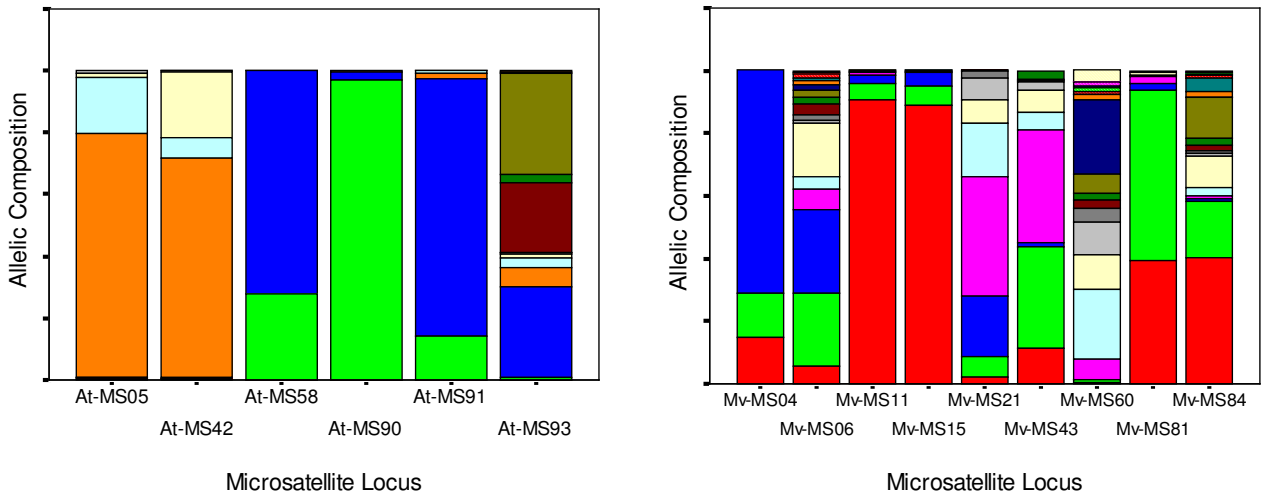

Fig. 54: Allelic Composition of microsatellite loci in *A. transversus* (left) and *M. vincta* (right).

relatively invariable in comparison to the hyper variable system of *M. vincta*, which contains more than twice the amount of alleles. The high variability of the latter poses problems in the analysis on the population genetic level, because the large number of alleles requires an adequate sample size of individuals per population, which was not given for *M. vincta*. However, it has been shown that cross amplifications of the established microsatellite system in closely related species show positive and polymorphic amplification products, which is promising for further analyses of the variable species group of the genus *Monolepta* (PATT *et al. in press*).

5.1.2.2. Deficiencies of Hardy-Weinberg Proportion and Null Alleles

Deficiencies of Hardy-Weinberg-Equilibrium were found for both microsatellite systems of *A. transversus* and *M. vincta*. Heterozygote deficiencies can be produced by biological as well as artificial reasons. Biological causes for an investigated excess of homozygote genotypes contain the possibility of WAHLUND-Effects, assortative mating or inbreeding (HARTL & CLARK 1997). Also parthenogenesis, which is reported for several species of the Curculionidae, is expected to result in a similar pattern (CLARKE 2000). However, asexuality is mostly connected with polyploidy (SUOMALAINEN & SAURA 1973, MESAROŠ & TUCIĆ 1995), while the observed mating behaviour and the diploidy of *A. transversus* do not indicate parthenogenesis in this species. Deficits in the number of heterozygotes have been already reported in a variety of studies concerning the population genetic structure of beetles and were discussed in different ways. CROUAU-ROY (1988) found a lack of heterozygotes over nine polymorphic loci in allozyme studies on cave-dwelling beetles of the genus *Speonomus*. As the pattern was represented across all investigated markers, she interpreted the result as caused by inbreeding. BILTON (1992) found a similar pattern in a study on the dytiscid beetle *Hydroporus glabriusculus* and attributed it to the sampling procedure conducted over several different aggregations. Heterozygote deficiencies in two leaf beetle species *Oreina cacaliae* and *O. speciosissima* led KNOLL & ROWELL-RAHIER (1998) to the assumption that inbreeding has produced kinship groups and a sampling effect over several different closely related demes was responsible for the pattern. However, it is expected that the mentioned biological reasons result in a pattern of heterozygous deficiencies, which is equally distributed across all investigated markers. LIEWLAKSANEEYANAWIN *et al*. (2001) found heterozygote deficiencies in four of five microsatellite markers in the white pine weevil *Pissodes strobi*. They could prove that

artificial reasons caused the observed pattern as the mentioned loci indicated the presence of null alleles in analyses of control crosses.

Both, *A. transversus* and *M. vincta* show Hardy-Weinberg-Equilibrium in at least one marker. The conformation to Hardy-Weinberg expectations of At-MS42 and Mv-MS21 as well as the occurrence of null homozygotes at most of the remaining loci are important indications for the presence of null alleles, while biological causes for the given result can be ruled out. Just a heterosis effect on the two concerning loci would produce a similar pattern, which is rather unlikely, as microsatellites are supposed to be selectively neutral (SCHLÖTTERER & WIEHE 1999). In the present study the proportion of null alleles was re-estimated and most of the analyses were calculated with the original as well as the corrected data set. While results of *A. transversus* were not significantly biased by the presence of null alleles, the results of *M. vincta* show critical changes under consideration of the re-estimated null allele matrix. As the data set of *M. vincta* was insufficient it was decided to omit several analyses and conducted tests concentrate on a basic characterization of the marker system and the populations of this species.

Although the null alleles clearly left a foot-print on the pattern of heterozygote deficiencies in the present study, the best way to check for null alleles is to examine the inheritance of alleles in known pedigrees. If possible, it should be considered to directly include such controls during the establishment of microsatellite systems. If a pedigree analysis is difficult, it is suggested to invest in designing and testing a number of primer pair combinations for each putative locus, because most null alleles arise from mutations in the primer binding sites (CALLEN *et al*. 1993, PAETKAU & STROBECK 1995). In fact, it is a time-consuming and even expensive option to use different primer sets for every locus – but it can be expected that these together will amplify most alleles.

5.2. The Genetic Structure of Amphitmetus transversus

5.2.1. Genetic Diversity

The genetic diversity inferred from the established microsatellite system of *A. transversus* ranges between 1.95 (Kai) to 3.16 (IseI) in case of allelic richness and from 0.223 (MaO) to 0.444 (IseI) in case of expected heterozygosity. In comparison to the variability of microsatellite systems in other beetles the observed diversity is rather low (e.g. BATLEY *et al.*

1998, BROUAT *et al.* 2003, DHUYVETTER *et al*. 2004, GARNIER *et al*. 2004). A considerable low variability has been found in a study on bark beetle species (Curculionidae) that was probably due to inbreeding effects in the two species (BERG *et al*. 2003), but is not reported generally in weevils (e.g. LIEWLAKSANEEYANAWIN *et al*. 2001, DHUYVETTER & DESENDER 2003, SALLÉ *et al*. 2003, GAUTHIER & RASPLUS 2004). However, the fact that different sets of microsatellites are used in the mentioned species renders the comparison of general polymorphism level in those populations difficult.

Regarding the population genetic structure of *A. transversus* it has to be considered that the Kakamega Forest and its adjacent fragments constitute the eastern range of the guineo-congolian rainforest complex and probably also the far outmost distribution range of *A. transversus*. Populations at the edge of the species distribution range often show a naturally reduced genetic variability (KRAUSS *et al.* 2004) due to a loss of genetic diversity because of bottlenecks during range expansion. The expansion of African rainforests after the last glacial period did not proceed in a single full fronted advance from West to East, but by colonising species establishing themselves as islands of woodland that later coalesced to form forests (MITCHELL 2004). From this view, it is possible that populations of the apterous and therefore low mobile *A. transversus* have undergone a bottleneck during range expansion, which has led to a generally low genetic diversity in the Kakamega Forest. Comparable genetic data from populations of the central parts of the guineo-congolian rainforest are necessary to confirm this hypothesis.

5.2.2. Genetic Differentiation among Populations

The geographic structure of a species or a set of populations on the genetic level is revealed by the distribution and abundance of genotypes within and among populations (RODERICK 1996). In the present study an attempt was made to discern the organization of microsatellite variation of the established marker system throughout the populations of the weevil *A. transversus* across the Kakamega Forest.

The weevil is distributed homogeneously across the Kakamega Forest. As the migratory potential of the apterous beetle is limited, dispersal preferentially occurs between geographically close populations, while genetic differences are inversely related to the amount of gene flow. A geographic variation of allele frequencies is therefore expected on the considered range of the study site. As shown in the geographic variation of allele frequencies the expectation was confirmed (Fig. 19–24). The pattern is influenced either by a varying

proportion of allele frequencies as well as by the occurrence of private or rare alleles, which are restricted to single or a small group of populations.

The geographical variation of allele frequencies is accompanied by a substantial genetic divergence among its populations as indicated by the significant values of F_{ST}. Examination of the raw data indicates that the high population differentiation was the result both of differences in allele frequencies across populations and the presence of rare alleles in certain populations. Based on the classification of WRIGHT (1931) *A. transversus* shows a moderate genetic differentiation among the collected populations on average. Approximately 12 % of genetic variation is found among sample sites. The value of F_{ST} is relatively high in comparison to values of microsatellite analyses reported for Carabids that have been sampled on a similar range (BROUAT *et al*. 2003, KELLER *et al.* 2004). Indeed, other studies of beetles show much higher values of F_{ST} (KING 1987, MESAROŠ & TUCIĆ 1995, DESENDER *et al*. 1998, GARNIER *et al.* 2004), but the different conditions render a comparison to the present analysis difficult. Those studies have investigated a larger geographical scale or beetles that naturally inhabit a patchy environment. Furthermore, some of the studies measured the amount of genetic differentiation using allozyme markers. DHUYVETTER *et al.* (2004) calculated F_{ST} by allozymes as well as by microsatellites for identical populations and could show that the value of genetic differentiation was considerable higher if measured with allozymes than with microsatellites. Therefore, a comparison of values of F_{ST} obtained by allozyme analysis to those measured with microsatellites should always be treated with caution.

The F_{ST} values of each marker are significantly different from zero, but considerably heterogeneous (from 0.043 in At-MS93 to 0.444 in At-MS05, Table 19). The highest values of genetic differentiation are given by At-MS05 and At-MS91. These are also markers, which show a considerable allelic variation on the geographical scale.

Pairwise genetic differences measured as F_{ST} reveal that most of the population pairs show a significant genetic differentiation. Nonetheless, several pairs did not show significant genetic differences. Several pairs of populations found within the northern part of the forest show considerable similarity despite geographic distances up to 15.21 km. This part of the forest does not even show an ‘isolation by distance’ pattern, although it includes at least 13 of the investigated populations that were sampled across a geographical area of approximately 120 km². The result suggests that geographical distance can not really account for the observed differentiation between populations. This is evident by the comparison of populations of the northern part of the continuous forest with those of the fragments Kisere and Malawa respectively. The genetic differentiation between the northern populations and

those sampled at Kisere is much higher than between the northern populations and those of Malawa, despite larger geographical distances towards the latter. However, regarding the whole investigated area of the Kakamega Forest a significant pattern of 'isolation by distance' has been found, which is probably due to the larger genetic differentiation between the northern and the southern populations that also show large geographical distances.

Beside of isolation by distance other physical or ecological barriers cause the genetic structuring of the weevil. Strong evidence was found for a genetic barrier between the southern fragment Kaimosi and the remaining populations, which is also impressively reflected in the allele frequency distribution. A second barrier is likely between the southern part and the northern part of the forest, as indicated in the analysis of NEI's genetic distance and the Monmonier's maximum difference analysis. Regarding the geographic distribution of allele frequencies this barrier is above all indicated in the allelic distribution of the marker At-MS05. The special situation concerning this topic is discussed later.

Interestingly, this study indicates that effects of the landscape on population genetic structure are concurrent with a significant pattern of 'isolation by distance'. Other studies found isolation by distance only in regions where habitat was continuous, while regions that exhibit genetic barriers did not reveal a significant correlation of genetic and geographic distance (BRITTEN *et al.* 1995, JOHNSON & BLACK 1995, LEBLOIS *et al.* 2000, HUTCHINSON & TEMPLETON 1999, SUMNER *et al.* 2004). They claim that if other landscape features than geographical distance influence genetic differentiation, isolation by distance is not detected. However, the present result shows that the arrangement of genetic composition due to physical or ecological barriers do not necessarily rule out the possibility of an detectable isolation by distance pattern (KEYGHOBADI *et al.* 1999).

In conclusion, the results showed that the investigated populations of *A. transversus* are genetically structured on the examined spatial scale of the present study. The geographical distance between sample sites explains the pattern partly by 'isolation by distance'. A more detailed analysis of the population genetic structure revealed that gene flow is also restricted by additional reasons, which are discussed in the following.

5.2.3. Effects of Anthropogenic Habitat Fragmentation and Degradation

5.2.3.1. Effects of Fragmentation on the Genetic Diversity

Theory predicts that the isolation of populations has two main genetic consequences (TEMPLETON *et al*. 1990). First, genetic differentiation between populations increases due to a limited or absent rate of migration. Second, a loss of genetic diversity is expected within small populations due to increased drift and inbreeding, which depends on the effective population size of the bottlenecked population. As a result of the inverse relationship between genetic drift and effective population size, it is expected that these effects should be first detectable in smaller populations, while larger populations may remain undifferentiated even if they are completely isolated from each other. An increase of genetic differentiation between isolated populations of beetles due to roads (KELLER & LARGIADÈR 2003, KELLER *et al.* 2004) or non-forested areas (BROUAT *et al*. 2003) has been reported in several studies located in the temperate climate, but these studies have not shown a detectable reduction in genetic diversity. KELLER *et al.* (2004) gives two possible explanations for the failure to detect a population bottleneck, despite large genetic differentiation between isolated populations. The first explanation claims that the power of the test on the basis of the given microsatellite set is weak, while the second concerns the biological possibility that the effective population size was not reduced to extremely low levels by fragmentation.

Generally, it has been reported in several studies that heterozygosity has a weaker resolution as a measure of genetic diversity compared to allelic diversity. LEBERG (1992) found that heterozygosity only weakly reflects a population's history of bottlenecks in analyses of allozymes. Similar results were obtained for microsatellites (SPENCER *et al.* 2000). This result is concordant with the theoretically expectations as gene diversity is less sensitive to a reduction in population size during a bottleneck than the number of alleles. A loss of alleles largely depends on the effective size of the bottlenecked population. In comparison, the amount of reduction in heterozygosity depends not only on the bottleneck size but also on the duration of the bottleneck or rather the generation time and population growth after going through a bottleneck. A bottleneck eliminates many low frequency alleles, while the remaining alleles still may exist at intermediate gene frequency. Hence, heterozygosity may not decrease substantially unless a decrease in population size is maintained for generations (NEI *et al.* 1975, ALLENDORF 1986).

The results of the present study indicate that the populations of the weevil *A. transversus* in the fragmented rainforest Kakamega Forest exhibit changes in genetic diversity, which can be attributed to an alteration of the habitat as predicted by theory. A comparison of populations located in fragments to those located in the continuous forest did not reveal large differences, but a decrease of genetic diversity at locus At-MS93 as well as lower frequencies of rare alleles in the fragments. However, the investigated fragments of the Kakamega Forest are of largely different quality. Kaimosi and Malawa have experienced massive deforestation and plantation during the last century and are of considerable small size, while the moderate fragment Kisere is the best preserved area of the forest. In consideration of these differences it was found that three fragments (Kaimosi, Malawa East and Malawa West) of sizes smaller than 200 ha (SF) show significant decreases in genetic variability in several tests.

A significant decrease of allelic richness in small fragments (SF) compared to continuous forest sites as well as to populations within the moderate fragment (MF) was observed. In addition, a significant correlation of allelic richness to the size of the fragment and the continuous forest respectively indicates a strong relationship between fragment size and the number of alleles. The pattern suggests a dependence of forest size on the number of alleles. Tendencies in the same direction were found for gene diversity. These results are theoretically consistent since the effects of fragmentation on the genetic diversity should be less evident in the parameter of gene diversity compared to allelic richness.

Differences for the proportion of rare alleles between continuous forest and fragments approach significance indicating that alleles at low frequency are less frequent in populations of fragments than of continuous forest sites (Fig. 35). This might be a result of a faster loss of rare alleles in fragments due to random genetic drift. The observed mode-shift in small fragments based on the corrected data set indicates that those populations are not in mutation-drift equilibrium, but have undergone a bottleneck recently (LUIKART & CORNUET 1998, Fig. 38). An indication of a decreasing genetic diversity of small fragments was not found in the number of polymorphic loci. Although this was lower in small fragments the result is not significant, which might reflect the inadequacy of microsatellites marker for the calculation of this parameter. Although LEBERG (1992) examined that the proportion of polymorphic loci often reflects a population's history of a bottleneck for allozyme data SPENCER *et al.* (2000) did not find similar results for microsatellites. He noted that the high variability of the marker might be responsible for the result.

The obtained results indicate a reduced genetic variability in populations of *A. transversus*, which might have been resulted from a reduction in population size. The lower genetic variability found in fragments of size smaller than 200 ha gives evidence of an effect of habitat fragmentation on the genetic diversity of the beetle. The examination of current habitat fragmentation always implies the assumption that the genetic variability of the considered population was similar prior to rainforest fragmentation. However, a reduced genetic diversity in isolated populations also can be historically low and must not necessarily be caused by the recent anthropogenic induced degradation of the fragments. MILLER & WAITS (2003) found in a temporal examination of genetic diversity of the Yellowstone grizzly evidence for historically low genetic diversity in separated populations and concluded that low levels of genetic diversity in an extant population may not be a strong evidence of a recent bottleneck. As we do not have information about the status of fragmentation prior to human activity, we don't have a clear idea, when the isolation of the northern fragment Malawa took place. As far back as records extend, Malawa was already separated from the main forest. For this reason we can assume, that Malawa was separated from the main forest not later than 1910. It was differently discussed at which time the fragmentation of the northern fragments occurred. MITCHELL (2004) suggests the possibility that the northern fragments have never been fully joined to the main part of the forest since the last forest expansion, while several other authors maintain the assumption that the fragmentation is man made and does not reach back more than 150 years (MUTANGAH *et al*. 1992, BLEHER *et al*. 2004). Indeed, the high genetic similarity of the beetle populations of the fragment Malawa to those of the northern part the forest makes a separation of the populations for thousands of years questionable. Kaimosi, on the other hand, was connected with the southern part of the continuous forest between 50 and 80 years ago and can therefore not be interpreted as a historically isolated population. The generally low genetic variability in three fragments smaller than 200 ha as well as the correlation between fragment size and allelic richness point out that the fragmentation and degradation of the Kakamega Forest has more likely an effect on the genetic diversity of the beetles than any other historical process.

The observed genetic pattern can be interpreted as a consequence of an extensive reduction of the effective population size in populations of fragments smaller than 200 ha. However, the limited mobility and presumed low habitat requirements of *A. transversus* are most likely not affected by the still given fragment sizes. An important fact is that the small fragments did not only have been reduced in size but also had to bear large impacts by deforestation and thus habitat changes during the last century. Actually, it has been shown in

several studies that habitat loss often has much larger effect on biodiversity measures than habitat fragmentation per se (FAHRIG 2003). The area of the small forests in the Kakamega Forest are likely to have been largely cleared and reforested during the last century down to the present day (MITCHELL 2004). As far as that goes the result may reflect a large reduction in population size of *A. transversus* due to deforestation and degradation of the concerned areas, whereas the genetic resources of the remnant populations were impoverished. As the isolation of the patches prevents migration from other populations the genetic diversity of the bottlenecked population could not be restored. The absence of significant declines of variation in moderate fragment populations, when compared to continuous forest populations, might be explained by the healthy state of the fragment Kisere that hold up genetic diversity and also supports the assumption that not habitat fragmentation per se, but habitat degradation and loss affect the genetic diversity of *A. transversus*. The populations of Kisere even reveal the highest values of allelic richness and gene diversity of the whole forest. Human impact by deforestation and plantation is less in Kisere than in the most sites of the continuous forest (MITCHELL 2004, BLEHER *et al.* 2004). The high genetic variability obtained in populations of *A. transversus* in this fragment might reflect the preservation status of the forest.

5.2.3.2. Contrast of Differences in Genetic Diversity at Single Markers: Evidence of Genetic Drift or Selection?

The analysis of differences in genetic diversity of single markers shows that the mentioned results are mainly based on two markers, At-MS91 and At-MS93. Two explanations can be considered. First, as the results are not evenly distributed across all markers the selective neutrality of the two markers might not be given and rather selection and less so genetic drift plays an important role in shaping the presented population structure. Alternatively, the two markers At-MS91 and At-MS93 contain more information than the others due to a higher variability.

At-MS93 is the most variable locus of the given data set with an average gene diversity of 0.71 and allelic richness of 5.95, while the others contain diversities between 0.05 and 0.42 for gene diversity and from 1.58 to 3.26 for allelic richness. Hence, At-MS93 also contains the highest proportion of rare alleles. Rare alleles are particularly affected by drift effects on allelic diversity (GARZA & WILLIAMSON 2001, CORNUET & LUIKART 1996, LUIKART *et al.* 1998). The higher sensitivity of this marker compared to the others is therefore not astonishing. On the other extreme, At-MS90 has the lowest values for allelic richness and gene diversity and shows the lowest variability with monomorphic occurrence in eleven

populations. Its power concerning the variability of allelic richness and gene diversity in different groups of populations is rather low. However, the gene diversity of At-MS91 is comparable to the other three markers (At-MS05, At-MS42, At-MS58). It shows a gene diversity of $H_S = 0.21$ and allelic richness of $\hat{A} = 2.47$. The big discriminative power of At-MS91 concerning differences in allelic richness and gene diversity can not be explained by its variability. The high resolution of this locus must be accidental or due to a selective effect on this marker. Microsatellites can be regarded as neutral markers, which are randomly distributed over the euchromatic part of the genome. However, directional selection at a linked locus may cause a deviation of microsatellite variation from their neutral expectations ("selective sweep") (SCHLÖTTERER & WIEHE 2001). While the locus under selection is being fixed throughout the population, the polymorphism in the flanking microsatellite may be wiped out, which leads to a reduction in genetic variation in this region. Although selective sweeps are rare, the possibility can not be ruled out for the given marker. Assuming that At-MS91 does not fit the requirement of neutrality, the test for differences in allelic richness was repeated between the continuous forest (CF), the moderate fragment Kisere (MF) and the group of the small fragments Kaimosi and both at Malawa (SF). The test revealed significant results for differences between CF and SF, likewise, although the level of significance was slightly lower.

5.2.3.3. Effect of Fragmentation on the Genetic Differentiation

Following theoretical expectations the genetic differentiation between fragmented populations increases due to a limited or absent rate of migration. However, a correlation of geographical isolation by fragmentation between pairs of populations on of the extent of genetic differentiation between pairs of populations of *A. transversus* did not show a significant result in a Mantel test. The result indicates that the fragmentation of the Kakamega Forest does not increase genetic differentiation between populations of fragmented sites.

The result is confirmed when genetic differences between pairs of populations are compared separately for each fragment. The fragment Malawa is only weakly differentiated from most of the populations of the northern part of the continuous forest. The similar genetic structure is reflected in the distribution of allele frequencies as well as some missing significances of pairwise genetic differences measured as F_{ST} between pairs of populations of this part of the forest. Populations located at the fragment Kisere exhibit significant genetic differences towards the populations located at the northern part of the continuous forest. Nevertheless, significant differences are missing between some pairs of populations of Kisere

and Malawa as well as of Kisere and Isecheno I, despite of their geographical separation by less suitable landscape elements and a supposed lack of migration. In contrast to the northern fragments the genetic differentiation between the southern fragment Kaimosi and the remnant populations is extremely high. Kaimosi reveals the highest values of pairwise F_{ST} towards all other populations of the Kakamega Forest. This result is also reflected in the isolated position of Kaimosi in the phenogram as well as in the outcomes of the tests on genetic barriers.

Although the fragment Kaimosi is clearly differentiated from the remaining populations a general effect of anthropogenic fragmentation on the population genetic differentiation could not be confirmed, as the genetic constitution of the populations in the other fragments shows.

5.2.3.4. Conclusion

The data of genetic diversity in *A. transversus* indicate that fragmentation and the degradation of the Kakamega Forest have an effect on the genetic diversity of the *A. transversus* populations. Small and largely degraded fragments reveal significantly lower genetic diversity relative to the moderately sized fragment and continuous forest sites. The prevalence of a mode shift in small fragments supports this result. However, the genetic differentiation between fragmented populations has not been significantly increased in general. Kaimosi, which exhibits the smallest genetic diversity, shows an immense increase in genetic differentiation towards the remaining populations, while Malawa has not undergone great allele frequency changes, despite its demonstrable small genetic diversity. This leads to the conclusion that the different constitution of the population at Kaimosi is probably not exclusively based on the fragmentation effect, but also affected by other mechanisms. An examination of effects of the current fragmentation process implies the assumption that the amount and distribution of genetic variation in the considered areas of continuous and fragmented sites were similar prior to rainforest clearing. The genetic characteristics of the fragment Kaimosi might be an indication that this was not the case in the Kakamega Forest. However, a repeated analysis on differences in allelic richness without the fragment Kaimosi was still significant.

From the present state it can be concluded that the results are most probably caused by the effect of random genetic drift on the genetic variability of *A. transversus* in smaller and more affected habitats of the Kakamega Forest. Habitat loss and fragmentation even has an effect on a low mobile invertebrate, such as *A. transversus*. The beetle obviously suffers from the massive degradation of the small fragments, while beetles of the moderate fragment

Kisere are not affected by the loss of genetic variability. It has already been shown in conservation studies on invertebrates that the reduction of area size to 100 ha and less has a considerable influence on invertebrate population density, species richness and species composition and that these fragments' faunas are distinct from undisturbed continuous forest due to the local extinction of some dominant species and an unknown number of rare species (DIDHAM 1997). Authors claim that not only an isolation and reduction of the fragment area is responsible for this result, but also the increasing edge effects with decreasing fragment size (DIDHAM 1997, MAGURA *et al*. 2001, BARBOSA & MARQUET 2002). Derived from the present results it seems that a minimum size of fragments is also needed to obtain genetic diversity of single species at a native level that can be compared to habitats not affected by fragmentation and degradation.

5.2.4. The Special Case of the Fragment Kaimosi

The most impressive result of the present study is the genetic constitution of the southern fragment Kaimosi, which differs from all other populations of the forest in many respects.

The high F_{ST} value between Kaimosi (Kai) and Kibiri (Kib) suggests that gene flow between these parts is historically restricted. An assumption which is also confirmed by the high proportion of a private allele at Kaimosi. Microsatellites are characterized by a high mutation rate and hence, a large polymorphism. Due to the large number of alleles usually a certain number of private alleles exist. Actually, private alleles can be found for each marker. Most of them exist at low frequencies ($p \leq 0.1$), with the exception of the private allele '18' of At-MS05 which occurs in the fragment Kaimosi. This allele exists in a considerable high proportion ($p = 0.18$). Rare alleles are unlikely to be included in migrant organisms unless the migration rate between populations is high and they tend to remain present in only one or a few subpopulations in a local area (HARTL & CLARK 1997). However, the allele '18' of Kaimosi is present at an intermediate frequency and would be expected to be dispersed by migration. As this is not the case it can be deduced that migration is prevented between the fragment and the adjacent populations of the continuous forest.

The special allelic composition of this population is also shown in the allele frequency distribution of At-MS91. Beside of some rare alleles, the marker mainly consists of a common allele at high frequency at 18 sample sites. Kaimosi, on the other hand, is nearly fixed at an allele that was at low frequency in all other populations. KING (1987) found a similar genetic

dissimilarity for a population of the beetle *Collops georgianus* (Coleoptera, Melyridae) at the most western range of the species' distribution. She explained the structure by a historical event as the populations are relatively isolated from the others because they are at the edge of the species' distribution. However, the investigated scale of that study was much larger and the beetle showed a naturally patchy distribution. This is not the case for *A. transversus*, at least before human pressure fragmented the habitat into isolated patches. It has been documented in the forest's history that Kaimosi was connected to the continuous part of the Kakamega Forest at some time in the last century between 1913 and 1959 (MITCHELL 2004). It can be assumed that gene flow up to this time was not totally restricted to other investigated populations, but by geographical distance or natural barriers. In the case of the analysed population of Kibiri at the most southern part of the continuous forest the population of Kaimosi was even not separated by any detectable natural barrier before fragmentation.

If anthropogenic introduced fragmentation and degradation exclusively causes the observable pattern at Kaimosi we have to consequently ask why a similar extent of differentiation is not found for the other fragments, especially because Malawa and Kisere are separated earlier than Kaimosi (MITCHELL 2004). Even if Kisere is not taken into consideration, because it holds a special position due to its exceptional high genetic diversity, the different pattern of the small fragments Kaimosi and Malawa remains a puzzle. Assuming that the degradation and fragmentation gave rise to the large genetic differentiation of Kaimosi, this fragment had to be much more disturbed and deforested than Malawa. The effective population size of *A. transversus* must have been dropped substantially below the previous long-term average that random genetic drift had a strong effect on the bottlenecked population. An extreme bottleneck would explain the dramatic change of the allele frequency distribution at At-MS91 as well as the increasing proportion of the private allele '18' at At-MS05. The generally small genetic diversity found in Kaimosi, which was the smallest found in the whole area supports the assumption that the weevil populations at this site have undergone a strong bottleneck. Kaimosi is indeed the most disturbed fragment of the forest and has been nearly cleared during the last century (MITCHELL 2004). A similar impact is not reported for Malawa, although those fragments had to bear large human impact by deforestation and plantation, too. Nevertheless, it remains unclear, why Malawa, which shows a comparable low genetic diversity, does not demonstrate the expected genetic differentiation in a similar way. The most probable explanation is that fragmentation is not the only reason for the large genetic differentiation between Kaimosi and the remaining populations.

It has been reported that large parts of Kaimosi have been reforested and planted after the degradation of the forest. MITCHELL (2004) states that the present forest remnant is placed outside of the original forest cover of 1913/16 and is most likely a plantation. A bottleneck effect in combination with introduced beetle populations from distant habitats other than the Kakamega Forest during the extensive plantation would give a more plausible explanation for the large observed genetic differences. If we assume that the fragment was nearly destroyed during the last century and the population of inhabiting beetles almost vanished, then an introduced population could have caused a founder effect resulting in a small genetic variability but largely different genetic composition. Furthermore, the restriction of the beetles to the forest fragment prevented an expansion of the new introduced population, and hence of alleles, to other parts of the forest.

Besides the mentioned scenarios, additional explanations have to be considered, as the genetic distribution might be influenced by historical processes other than anthropogenic impact. As no obvious physical barrier has been detected between Kaimosi and the most southern part of the continuous forest site before human introduced fragmentation occurred, the low mobility of the beetle must have prevented the expansion of the private allele to adjacent populations (Kibiri). A theory which is generally discussed as a force of the diversification processes of tropical rainforests is the refugia model (MORITZ *et al*. 2000). It rests on the premise that climatic change caused shrinkage of rainforests to refugia separated by dry forests and savannah, which promoted speciation in the isolated habitats. Studies on this hypothesis in African rainforests are manifold (DIAMOND & HAMILTON 1980, BRÜHL 1997, FJELDSÅ & LOVETT 1997, ROY 1997). It has been proposed that montane areas in Africa acted as refugia and provided montane forest environments during the Quaternary. From this view a possible explanation for the observed pattern in the Kakamega Forest is that the current population of Kaimosi and the populations located in the North from Kaimosi derived from separate Pleistocene refugia. CUNNINGHAM & MORITZ (1998) found a similar pattern in the analysis of genetic effects of current fragmentation on a rainforest restricted lizard in Australia. The study took place on a micro geographical scale similar to that found at the Kakamega Forest. They could show that the effect of recent clearing on genetic differentiation appears minor compared to those from long-term climatic and geological processes and suggested that the analysed populations derived from separate refugia.

Unfortunately, there is no reliable information concerning the Pleistocene forest's history nor are biogeographically data of other rainforest-restricted species of the Kakamega Forest available to support this hypothesis. Nevertheless, the strong genetic differentiation

concerning the microsatellite markers and especially the private allele at intermediate frequency may indicate a dominant influence of natural rather than contemporary barriers to gene flow. A detailed analysis of the phylogeographical structure of *A. transversus* on mtDNA haplotypes might be informative in this respect. It was not possible to compare patterns of mtDNA variation among the populations, because of their general uniformity. Indeed, the yet conducted analyses did not include the population of Kaimosi. In order to test if Kaimosi actually constitutes a relic population of a different phylogeographical lineage a comprehensive analysis of mtDNA haplotypes as well as information about the existence and genetic composition of populations to the south of Kaimosi are unrenounceable. It would be helpful to investigate the South Nandi Forest, which was still connected with Kaimosi until the mid of the last century.

5.2.5. Genetic Dissimilarities between Northern and Southern Populations – Effects of Natural Barriers or Ecological Differentiation?

Strong genetic dissimilarities are found between parts of the forest apart from the extraordinary genetic constitution of Kaimosi. The spatial distribution of allele frequencies shows an obvious geographic pattern at several markers across the populations of the continuous forest. Allelic dissimilarities from the northern to the southern populations are indicated by the shifting allele frequencies at At-MS05. The allele ‘15’ occurs consequently in much higher proportion in the investigated populations of the southern part of the continuous forest, while the same allele is rare in the populations of the northern part (Fig. 19). The proportion of allele ‘15’ in all populations of Kisere (KiN, KiWW, KiS) as well as in two populations of Malawa (MalW, MalO) ranges between that of the northern and the southern populations. A characteristic similarity between the populations of the southern part of the continuous forest as well as the three populations of Kisere is also found in the allele frequency distribution of At-MS91 (Fig. 23). The allele ‘12’ occurs only in the three populations of Kisere as well as in two populations of the southern continuous forest, Yala (YalI) and Ikuywa (Iku). However, the allele frequency distribution of allele ‘22’ at At-MS93 reveals also similarities of a population at Kisere with two populations of the northern part of the continuous forest (IsiI & Cam, Fig. 24). The fragment Kisere exhibits several special features concerning the observed allele frequency distribution as it resembles populations of the south at several loci, but also reveals similarities to the northern populations. However, the

general pattern suggests among all a strong dissimilarity between the northern and the southern populations, which are located at areas that are currently connected by forest. The populations of Ikuywa (Iku), Yala I (YalI) and Kibiri (Kib) are located in separated parts of the rainforest but are still connected to the northern part by plantations. Field studies have shown that these forested areas are also inhabited by *A. transversus* and therefore the whole area can be interpreted as a connected habitat.

The changing genetic composition between the mentioned groups of populations has been confirmed by the test on genetic barriers. Following the outcome of the Monmonier's maximum difference algorithm, gene flow is limited between the northern and the southern part of the continuous forest. The genetic barrier was not as high as between Kaimosi and Kibiri but was supported to a high degree in the jack-knife analysis. A similar result was obtained using NEI's genetic distance. The population phenogram illustrates a large genetic distance between the northern and the southern group. A spatial analysis of variance (SAMOVA) did not support this result. This might reflect a weakness of the algorithm in the detection of barriers, as it searches for groups of populations that are geographically homogenous in the first place, while genetic barriers are revealed quasi as a by product (DUPANLOUP *et al*. 2002). Nevertheless, observations of allele frequencies as well as two further results on genetic distances support the high genetic dissimilarity between the northern and the southern populations.

Anthropogenic impact as a reason for the differentiation can be ruled out as the barrier is located in the middle of the continuous forest. The genetic structure of the population appears to be formed by historical or natural separation. Like every natural environment, the Kakamega Forest is structured.

5.2.5.1. Riverine Barriers

That rivers act as barriers to dispersal and hence are geographical causes of allopatric speciation has been realized since more than 50 years (MAYR 1942, 1963) and is discussed as a major force of diversification in tropical ecosystems (MORITZ 2000). Riverine barriers sometimes separate closely related species or races, but also populations of different phenotypes (e.g. POUNDS & JACKSON 1981). It has been found that rivers act as effective barriers in studies concerning beetles (KING 1987). The most effective ones being rivers that change their course least often, because of the long-term isolation of populations. Two major rivers pass the Kakamega Forest in the northern and the southern part of the continuous forest and are the most likely riverine barriers, which are expected to have been in existence for a

long time. The Isiukhu River has its source at the hills of the North Nandi Forests and passes the Fragment Kisere on the north-western edge. Along the south-eastern edge the fragment is passed by the smaller Nandamaywa River, which also has its source in the North Nandi Forest (Fig. 7). The fragment Kisere is therefore enclosed by two rivers and separated by the natural barriers from other sites of the forest. The Nandamaywa flows into the Isiukhu River right behind the fragment Kisere and before the Isiukhu enters the continuous forest, where it dissects the rainforest into a southern and a northern part. The second major river, the Yala passes the forest at the south and separates the populations of Kibiri and Kaimosi from the remaining populations.

Kisere obviously has a extraordinary position as it is surrounded by two rivers and separated from all other parts of the Kakamega Forest. This special feature is also represented in its allelic composition at several markers. Regarding the genetic composition it resembles the southern populations, but also shares common alleles with northern populations. If we take into consideration that the enclosing rivers are not that big near their sources and have not yet met at Kisere these might not be effective barriers and single individuals can be easily drifted across the streams. Moreover, the south-eastern edge is close to the continuous forest and hence to the southern part, if the Isiukhu River is considered as the border between the northern and the southern part of the forest. It is assumed that these parts are not separated for as long by human induced fragmentation as the north-western side. This would explain a higher similarity of the populations at Kisere to the southern than to the northern part of the forest. On the other hand neither does the test on genetic barriers nor the phenogram based on genetic distance indicate a stronger relationship of Kisere to the southern than to the northern populations. Although some barriers have been detected surrounding the Kisere population at the north (KiN) during the jack-knife analysis of the Monmonier's maximum difference algorithm, the border is only weakly supported and remains questionable.

The Yala River in the southern part of the forest separates the population of Kaimosi and Kibiri from the rest of the forest. Regarding the allelic composition of these populations Kaimosi has a special history anyway, while Kibiri shows some specific alleles but, in total, resembles the other populations of the south.

In a Mantel test, the hypothesis whether riverine barriers show a positive correlation to genetic differentiation was examined. Kisere was considered as an enclosed fragment, which is separated to all other populations by at least one river. The outcome of the simple Mantel test shows a high correlation parameter in combination with a high significance ($p < 0.001$).

However, with respect to the geographical distance, the result could not be maintained. On the other hand, geographical distance was not significant with respect to riverine barriers, either.

In conclusion, several reasons support the hypothesis that riverine barriers play an important role in the population structure of *A. transversus*. The strong dissimilarities between the North and the South have been shown in the phenogram as well as in the Monmonier's maximum difference algorithm (Fig. 41 and 42). A simple Mantel test also shows a positive correlation. The results indicate the presence of a barrier which fits to the Isiukhu River. However, the position of Kisere, which would be expected to be mostly differentiated due to its enclosed position, remains questionable and can only be explained by a partial gene flow due to drifted individuals across the streams at this site. Furthermore, the result of the simple Mantel test could not be confirmed when controlling for geographical distance. Finally, riverine barriers seem not to act generally as barriers because a similar pattern is not found for the Yala River, which is similar in size. Generally little is known about the geology of the two river systems at the Kakamega Forest and accurate information is necessary to explain the whole pattern.

5.2.5.2. Ecological Differentiation

The Kakamega Forest is ecologically structured, which is, among other things, reflected in soil structure as well as the composition of vegetation. The ecological differences extend from the northern part to the southern part of the forest, but the areas of differentiation in vegetation and soil structure are not congruent. If the causes of the mentioned ecological differentiation also plays a role in the populations of *Amphitmetus transversus* it is expected that individuals inhabiting the same ecological area are genetically more similar than those of different areas.

It has been reported for several monophagous insects that a specialization on varying feeding plants has an effect on the amount of genetic differentiation and it has been proved that host plants can play a major role in isolating specialized populations via unique selection pressures, leading to the formation of host races (e.g. MCCAULEY 1991, RANK 1992, KERDELHUÉ *et al*. 2002). RANK (1992) states in his study on leaf beetles that the association with patchily distributed host plants that show phenological differences has important consequences for the subsequent evolution of herbivores insects whose vagility is limited.

A specialization in a similar way has not been detected for *A. transversus* during the field work. The weevil is a generalist and feeds on a large variety of vascular plants. However, it can not be neglected that a phytophagous species lives in close association to the

hostplant and possibly depends on the composition of vegetation. ALTHOF (personal communication) found that the vegetation of the southern part of the Kakamega Forest differs characteristically from that in the northern part. The altering composition of the vegetation is caused by changing environmental conditions like the amount of precipitation, the altitude and the average temperature. The shifting ecological parameters, which are reflected in the changing vegetation, may also be reflected in the genetic differentiation of *A. transversus*. In many cases, local environmental variation causes natural selection to operate differently among local populations, and populations may differ genetically in response. Because of the naturally low vagility of the weevil the potential for local adaptation to small scale environmental variation might be enhanced.

Actually, it has been intensively discussed, if the large diversification of tropical ecosystems is rather based on the divergent selection across strong environmental gradients than on allopatric speciation promoted by the refugium model or riverine barriers (MORITZ *et al.* 2000). It is expected to result in sister species adapted to adjacent but distinct environments. The model suggests that strong environmental gradients resulted in differentiated adaptation and speciation and is supported by the frequent location of hybrid zones in ecotones (ENDLER 1982, ERWIN 1991). MORITZ *et al*. (2000) claimed that higher speciation rates in environments with strong habitat heterogeneity are consistent with the potential for speciation via diversifying selection.

Microsatellites are assumed to be selectively neutral and are not expected to show a pattern of adaption to different environments. Alternatively, if *A. transversus* is adapted to the differences in the environment, beetles of the same ecological ‘zone’ possibly mate more likely or have more mating success than that of different zones, which is reflected in a larger genetic differentiation between those groups.

The tested hypothesis in the simple Mantel test shows the highest correlation parameter, but the P-Value is rather low. The latter is due to the small number of samples involved in the analysis. Only those populations were taken into consideration that inhabited similar succession states of vegetation. While the hypothesis was significantly confirmed in the simple Mantel test the partial Mantel test remains influenced by the geographical distance. A simple ‘isolation by distance’ pattern can not be ruled out.

Larvae of weevils often live at the roots of their host plants (LAWRENCE & BRITTON 1991, BASSET 2001). As these insects are exposed directly to soil a varying composition of the habitat may result in a genetic differentiation of the beetles in a similar way as explained by the changing vegetation. The soil structure of the Kakamega Forest has been extensively

studied and shows a diverse composition. However, the outcome of the Mantel test concerning the soil structure was the weakest of the whole set. Although the result was highly significant, the correlation parameter was rather low. It is indicated that the soil structure constitutes a cause of genetic differentiation in the simple but not in the partial Mantel test.

5.2.5.3. Conclusion

Currently, we have no means to distinguish between the presented hypotheses. A methodical problem was the covering effect of isolation by distance, which prevents the clear examination of the hypotheses due to the given sampling in using the statistics of a partial Mantel test. Close sample sites tend to have similar environments, so that environmental and spatial distances will often be positively correlated. Furthermore, the geographical distance is expected to be correlated to the genetic distance ("isolation by distance"). Consequently, a positive association between environmental and genetic distances may be caused by spatial effects. Instead, also the opposite result is conceivable. Differences in the environment might mainly cause the genetic differentiation of the species, but due to the larger distances between those populations a simple 'isolation by distance' pattern is pretended.

No 'isolation by distance' pattern was detected between populations of *A. transversus* in the northern range of the forest, although great geographic distances could be recorded between similar populations comparable to most distances to the southern populations. Unfortunately, the large geographic distances between these population cluster does not clearly resolve the effects which are caused by geographic distance and those caused by other environmental factors. Populations in the centre of the forest would be most interesting in verifying the causes of genetic differentiation. On the present basis of the data set several hypothesis concerning environmental causes in population structure were significant in simple, but not in partial, Mantel tests. This means that effects of riverine barriers, different vegetation composition and soil structure on the population genetic structure of *A. transversus* could not be confirmed with respect to the geographical distance and the hypotheses remain to be tested with a more suitable sampling design. One would have needed populations of the centre as well as along both sides of the river to confirm if more likely environmental changes or geographic distance contribute to the genetic differentiation between the populations of *A. transversus*.

5.3. Genetic Structure of Monolepta vincta

5.3.1. Genetic Diversity

The genetic variability inferred from the established microsatellite system of *M. vincta* is rather high. In total, the markers show between three (Mv-MS04) and 20 (Mv-MS81) alleles and an expected heterozygosity of a wide range from 0.17 (Mv-MS 11) to 0.83 (Mv-MS06). Across all markers the mean expected heterozygosity per population was found to be between 0.49 and 0.61, while the number of alleles ranged between 2.78 and 7.78. The latter show a strong dependence on the number of individuals found in each population. The variability of the microsatellite system is comparable to those found for other beetle species (e.g. BATLEY *et al.* 1998, DHUYVETTER & DESENDER 2003, SALLÉ *et al.* 2003, GAUTHIER & RASPLUS 2004, KELLER *et al.* 2004) including also a study on leaf beetles (SEMBÈNE *et al.* 2003).

5.3.2. Genetic Differentiation among Populations

The variation of allele frequencies does not show a considerable geographical structure among the six investigated populations (Fig. 45–53). The high allelic variability of several markers makes an examination of the allele frequency distribution difficult. Although several rare alleles are clustered in regional groups a general pattern is not found. The weak geographic resolution that is illustrated by the allele frequency distribution is also obtained by the calculation of genetic differences measured as F_{ST} (Table 33). Pairwise F_{ST} estimates show genetic distances up to 2 % of variation between Isecheno (IseI) and Busumbuli (BusII). Although the genetic differentiation was significant for nine of 15 pairwise comparisons the extant of genetic differentiation is rather low (Table 34). The outcome of the F-statistics gives a total of 0.6 % of variation among the six populations. ‘Isolation by distance’ pattern was not detected by a simple Mantel test. The isolating effect of geographical distance depends on the gene flow between populations as well as on the influence of random genetic drift. The extent of the latter is inversely correlated to the effective population size, while the former corresponds to the rate of migration. Migration is related to the mobility of the concerning species and long range expansive species are less isolated by spatial dimensions due to ongoing gene flow. *M. vincta* is able to fly and shows a highly active behaviour. Therefore the small amount of genetic differentiation as well as the missing ‘isolation by distance’ pattern

can be explained by the extensive movement of individuals and the resulting gene flow among the investigated micro geographical scale of the present study.

On the other hand, the sample size of *Monolepta vincta* is quite small and only six populations could be analysed. The number of individuals in each population ranges from twelve to 41, which is comparatively low regarding the high number of alleles found at several markers. It can not be assumed that all alleles are represented in the samples. This is also indicated in the high dependence of allelic variability from sample sizes. The data set is probably biased and does not represent a sufficient sample of the given population. Concerning the re-estimated null allele matrix, the probability of biased results even increases. The estimated frequency of null alleles of the specific marker is rather high. It is likely that more than one allele has not been genotyped. However, in the re-estimated null-allele matrix only one null-allele is represented at a comparable high frequency. This leads to the conclusion that the obtained results might be partly artificial.

Although a genetic differentiation could not be proved on the scale and the sampling of the present study, the developed microsatellite system might be helpful on a larger geographical range. A recently published revision of *Monolepta vincta* has revealed the highly morphological polymorphism in colouration, which has – beside of the wide distribution and high abundance across the African continent – led to a high number of synonyms for the species (WAGNER, in press). A genetic analysis on the presented microsatellite system may be helpful to reveal the relationship within the highly diverse species on a larger geographical range.

5.4. Genetic Differentiation of *Amphitmetus transversus* vs. *Monolepta vincta*

Significant genetic differentiation was found between most of the sampled populations of *Amphitmetus transversus* and several sample sites of *Monolepta vincta*. The total amount of genetic differentiation across all populations, that was measured as F_{ST}, showed a moderate genetic differentiation of *A. transversus* populations with a variation of approximately 12 % across the Kakamega Forest and presented a significant 'isolation by distance' pattern, while *M. vincta* showed only a minimal differentiation of 0.06 % of variation, that could not be explained by the geographical distance between sample sites. The mentioned results can be interpreted as a consequence of the different mobility of the two species. *M. vincta* as a long range expansive species is expected to show a smaller extent of genetic differentiation than

the apterous *A. transversus* due to the higher rate of migration and hence, gene flow across the observed range. Nevertheless, a direct comparison of the obtained data remains difficult. As already mentioned, the variability of the established marker systems is quite different. Microsatellites of *M. vincta* showed a high diversity and the available data set might be too small for more detailed results. In total, 19 populations of *A. transversus* could be examined, but only six of *M. vincta*. Furthermore, there is only poor knowledge about biological and ecological properties of the tropical beetles and a simple comparison between the two species due to their different mobility remains incomplete.

5.5. Are Phytophagous Insects useful Indicators of Forest Change and Fragmentation on the Population Genetic Level?

The results of the genetic analyses on *Amphitmetus transversus* turned out to be notably yielding. The genetic population's structure of the weevil contained both information about contemporary as well as historical conditions of landscape structure. Regarding the human impact on the Kakamega Forest and its consequences some of the results predicted for increased population subdivision have been confirmed. The beetle populations in strongly degraded fragments of size smaller than 200 ha obviously suffer from habitat destruction, as tests on the reduced genetic diversity reveal. However, the outcome of genetic differentiation between populations suggests additional effects of long-term natural rainforest fragmentation. The results show impressively that effects of human activities on natural habitats in the recent past always have to be assessed in the context of long-term natural fragmentation. CUNNINGHAM & MORITZ (1998) already showed in the analysis of a rainforest restricted lizard that particularly tropical rainforest ecosystems are influenced by historical changes in rainforest distribution, which have a major impact of regional variation on a small geographical scale. The presented pattern of genetic diversity and differentiation might be generated by combinations of historical and ongoing processes and can not clearly be separated from one another, but the actual study can provide demographic and ecological hypotheses that can be tested with further field and molecular studies.

The detailed analysis of *A. transversus* could not be reproduced for *M. vincta*. The sampling of the leaf beetle turned out to be more difficult, which resulted in a smaller sample size. The microsatellite system was less informative on the given geographical scale which

was probably caused either by the small sampling as well as by the large mobility of the beetle. This leads to the assumption that especially invertebrates that are less or moderate mobile are useful in an analysis of the effect of landscape structure at the micro geographical scale like in the present study.

The possibility of collecting a representative number of samples is undoubtedly an advantage of population genetic studies on invertebrates. A comprising study on a vertebrate that is comparable to the conducted study on *A. transversus* needs probably much more effort. A problem in detailed analysis of invertebrates in tropical rainforest systems is the difficulty in achieving basic biological and ecological data. I am not aware of any study that has examined the impact of habitat fragmentation and degradation on an invertebrate species in the tropical rainforest yet. The lack of this data is unfortunate as studies involving invertebrates have already proved the considerable potential of insects and especially beetles in investigating and testing hypotheses of the demographic and genetic impacts of fragmentation (e. g. DESENDER *et al*. 1998, CLARKE 2000, KRAUSS *et al*. 2004, KELLER *et al.* 2004). The relatively small spatial scale and rapid generation time of beetles and other invertebrates make them useful in the analysis of human impact on a micro geographical scale. That insects can be treated as indicators in this regard has been demonstrated various times and could be confirmed in this study concerning changes in landscape structure of a tropical ecosystem. The results indicate that the weevil *A. transversus* is affected by forest change, despite its suggested small scale habitat requirements. Although this study focussed on the potential use of genetic data for inferring demographic parameters and for understanding past and recent population processes it must be stressed that genetic data alone should not be viewed as being either comprehensive or exclusive. However, the study shows that genetic surveys are an extremely useful tool for identifying pattern and addressing consequences of habitat fragmentation. For a comprehensive understanding of the dynamics of invertebrates in tropical ecosystems an integration of studies from genetics, ecology and life history are highly needed. Given the prominence of tropical arthropods in accounts of global and tropical species diversity, the poor effort in attaining a basic scientific knowledge on species' natural history and ecology is clearly a major omission. Despite their importance for ecosystem function, the inconspicuous group of the insects unfortunately remains largely unknown.

6. Literature

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7. Appendix

Table A1: Original genotype matrix of *Amphitmetus transversus.* Missing genotypes indicated as '0000'.

	At-MS05	At-MS42	At-MS58	At-MS90	At-MS91	At-MS93
Col						
	1414	1414	0909	1010	1011	1923
	1414	1414	0909	1011	1111	0623
	1414	1414	0909	1011	1111	0707
	1414	1416	0909	1010	1111	1919
	1414	1415	0909	1010	1111	0723
	1414	1416	0909	1010	1111	0619
	1414	1516	0909	1010	1010	0606
	1414	1416	0909	1010	1111	0606
	1414	1516	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0619
	1414	1415	0909	1010	1111	0606
	1414	1414	0909	1010	1111	1919
	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0623
	1414	1515	0808	1010	1111	0000
	1414	1415	0909	1010	1111	1919
	1414	1414	0909	1010	1111	0619
	1414	1414	0909	1010	1111	1919
	1414	1414	0909	1010	1111	0606
	1414	1416	0909	1010	1111	1919
	1616	0000	0000	0000	0000	1923
	1414	1616	0909	1010	1111	1922
	1414	1414	0909	1010	1111	1919
	0000	0000	0303	1010	0000	0000
	1414	1414	0000	1010	1011	2323
	1414	1416	0809	1010	1109	0719
	1414	1416	0808	1010	1111	0619
	1414	1414	0909	1010	1111	0723
	1414	1414	0809	1010	1011	0607
	1414	1416	0909	1010	1111	1923
	1414	1416	0808	1010	1111	0606
	1414	1510	0909	1010	1111	0623
	1414	1414	0909	1010	1111	1923
	1414	1415	0909	1010	1011	1923
	1414	1414	0909	1010	1111	0623
	1414	1410	0909	1010	1111	0623
	1414	1414	0808	1010	1111	0619
	1414	1414	0809	1010	1111	1923
	1414	1415	0909	1010	1111	0619
	1414	1414	0808	1010	1011	2323
	1414	1416	0808	1010	1115	0719
	1414	1416	0909	1010	1111	0623
	1414	1414	0808	1010	1111	0619
	1414	1415	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0623
	1414	1414	0909	1010	1011	0619
	1414	1414	0909	1010	1111	0606
	1414	1415	0909	1010	1011	0619
	1414	1414	0909	1010	1111	0619
	1414	1414	0808	1010	1011	0619
BusI						
	1414	1516	0909	1010	1011	1923
	1414	1416	0909	1010	1111	0619
	1414	1415	0808	1010	1011	0623
	1414	1717	0909	1010	1111	0608

	1414	1414	0909	1010	1011	0709
	1414	1415	0909	1010	1111	0607
	1414	1415	0909	1010	1111	0607
	1414	1415	0909	1010	1011	0623
	1414	1516	0808	1010	1011	1923
	1414	1414	0909	1010	1111	0606
	1414	1416	0909	1010	1111	0623
	1414	1414	0909	1010	1111	0619
	1415	1415	0909	1010	1011	0606
	1414	1415	0909	1010	1011	0607
	1414	1414	0909	1010	1111	1919
	1414	1415	0808	1010	1111	1923
	1414	1414	0808	1010	1111	1919
	1414	1414	0909	1010	1011	0623
	1414	1414	0909	1010	1111	1923
	1414	1416	0909	1011	1011	0623
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1011	0623
	1414	1616	0909	1010	1111	0606
	1414	1616	0909	1010	1111	0606
	1414	1415	0909	1010	1111	0619
	1414	1414	0909	1010	1111	2323
	1414	1414	0808	1010	1111	0619
	1414	1416	0909	1010	1111	1923
	1414	1415	0909	1010	1011	2323
	1414	1415	0909	1010	1011	0606
Kai						
	1414	1416	0909	1010	1010	1919
	1418	1414	0909	1010	1010	1923
	1414	1414	0909	1010	1010	1919
	1414	1414	0909	1010	1010	1923
	1414	1414	0909	1010	1010	1923
	1414	1414	0909	1010	1010	1923
	1414	1416	0808	1010	1010	1923
	1414	1416	0909	1010	1010	2323
	1414	1414	0909	1010	1010	2323
	1818	1414	0909	1010	1010	0619
	1414	1416	0909	1010	1011	2323
	1414	1414	0909	1010	1010	0623
	1818	1414	0909	1010	1010	1923
	1414	1416	0909	1010	1010	1919
	1414	1414	0909	1010	1010	0623
	1414	1416	0909	1010	1010	0623
	1414	1414	0909	1010	1010	1919
	1414	1414	0909	1010	1010	0606
	1818	1414	0909	1010	1010	2323
Iku						
	1616	1414	0808	1010	1212	1919
	1515	1416	0808	1010	1111	0619
	1515	1416	0909	1010	1010	0605
	1515	1415	0808	1010	1111	2323
	1515	1416	0808	1010	1111	0919
	1415	1416	0808	1010	1011	0619
	1415	1415	0808	1010	1111	2323
	1414	1516	0808	1010	1010	0623
	1515	1414	0909	1010	1011	0623
	1515	1415	0909	1010	1212	0623
	1515	1414	0808	1010	1111	1923
	1515	1416	0909	1010	1111	0605
	1515	1416	0808	1010	1011	1923
	1515	1415	0909	1010	1111	0619
	1414	1414	0808	1010	1111	0606
	1515	1414	0808	1010	1011	0606
	1515	1416	0808	1010	1111	0608

	1515	1414	0909	1010	1111	0623
	1516	1416	0909	1010	1111	0708
	1515	1416	0909	1010	1111	1924
	1414	1414	0909	1010	1111	0606
	1515	1416	0909	1010	1111	0719
	1515	1414	0808	1010	1111	1919
Buk						
	1414	1414	0808	1010	1111	2323
	1414	1414	0909	1010	1111	0719
	1515	1416	0909	1010	1011	0619
	1414	1414	0909	1010	1111	0623
	1414	1416	0909	1010	1111	0723
	1414	1412	0808	1010	1111	1923
	1616	1412	0909	1010	1111	0623
	1414	1414	0808	1010	1111	0619
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0623
	1414	1414	0808	1010	1111	1923
	1414	1414	0909	1010	1111	2323
	1414	1414	0808	1010	1111	0623
	1414	1414	0909	1010	1111	0624
	1414	1414	0909	1010	1111	2323
	1416	1415	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0623
	1414	1410	0808	1010	1111	1923
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1111	0723
	1616	1414	0909	1010	1111	1923
	1415	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0619
	1414	1416	0909	1011	1111	0723
	1414	1414	0909	1010	1111	0623
	1414	1412	0909	1010	1111	2323
IsiI						
	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1010	1111	1919
	1414	1416	0909	1010	1111	0709
	1414	1416	0808	1010	1111	2222
	1414	1414	0909	1010	1111	1919
	1414	1414	0808	1010	1111	2222
	1414	1416	0909	1010	1111	2222
	1414	1416	0909	1010	1111	0608
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	1414	1416	0808	1010	1111	0606
	1414	1416	0909	1010	1011	0606
	1414	1416	0808	1010	1111	0819
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	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0606
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	1415	1414	0808	1010	1011	0709
	1414	1414	0909	1010	1111	0624
	1414	1414	0909	1010	1111	2323
	1414	1416	0909	1010	1111	0623
	1414	1416	0909	1010	1111	2323

	1414	1516	0909	1010	1111	0608
	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1010	1011	0909
	1414	1416	0808	1010	1011	1919
	1414	1416	0909	1010	1111	1923
KiN						
	1414	1414	0909	1010	1111	1923
	1414	1416	0909	1010	1011	1923
	1414	1416	0909	1010	1012	1919
	1414	1414	0808	1010	1011	1923
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	1414	1414	0808	1010	1011	1923
	1414	1416	0909	1010	1011	1919
	1414	1414	0808	1010	1111	0623
	1414	1416	0909	1010	1111	0608
	1515	1416	0808	1010	1113	0819
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	1415	1414	0909	1010	1011	0823
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	1414	1414	0808	1010	1111	1919
	1414	1414	0808	1010	1111	1919
KiWW						
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	1414	1414	0808	1010	1012	2323
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	1414	1416	0909	1010	1111	2323
KiS						
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	1414	1416	0909	1010	1111	0608
	1414	1416	0909	1010	1111	1919
	1515	1416	0909	1010	1109	0606
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	1414	1616	0000	1010	1011	0608
	1415	1414	0909	1010	1011	0622
	1414	1416	0909	1111	1010	1922
	1414	1416	0909	1010	1011	0622
	1414	1416	0808	1010	1113	0619
	1414	1414	0808	1010	1111	0623
	1414	1416	0909	1010	1111	2222
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	1414	1416	0909	1010	1111	1919
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	1414	1416	0909	1011	1111	0608
	1414	1414	0909	1010	1111	0606
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	1414	1416	0909	1010	1212	2323
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	1414	1416	0909	1012	1111	0623
	1414	1516	0909	1010	1011	2323
	1414	1415	0909	1010	1011	0823
Vih						
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	1414	1416	0909	1010	1111	0606
	1515	1416	0808	1010	1111	2323
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	1414	1416	0909	1010	1111	0723
	1515	1416	0909	1010	1111	2323
	1515	1416	0808	1010	1111	1923
	1515	1414	0909	1010	1011	1818
	1414	1414	0909	1010	1111	0607
	1414	1414	0808	1010	1011	0607
	1515	1416	0909	1010	1111	0723
	1515	1416	0909	1010	1111	2323
	1515	1415	0909	1010	1111	2323
YalI						
	1515	1415	0909	1010	1111	0619
	1616	1416	0909	1010	1111	0607
	1515	1414	0909	1010	1111	2323
	1515	1417	0909	1010	1111	0623
	1515	1616	0909	1010	1011	2323
	1415	1414	0909	1010	1111	0623
	1515	1516	0909	1010	1111	1923
	1414	1414	0808	1010	1012	2323
	1515	1416	0909	1010	1111	1923
	1515	1616	0909	1010	1111	1923
	1515	1616	0909	1010	1011	0623
	1515	1416	0808	1010	1011	2323
	1515	1616	0808	1010	1112	2305
	1414	1416	0808	1010	1111	0610
	1515	1414	0808	1010	1111	1919
	1515	1616	0808	1010	1111	0623
	1515	1416	0909	1010	1111	1919

	1515	1416	0808	1010	1011	0723
	1515	1414	0808	1010	1010	0623
	1515	1416	0808	1010	1111	2323
	1515	1414	0909	1010	1111	2305
	1515	1416	0809	1010	1111	0623
	1515	1416	0909	1010	1111	0623
	1515	1416	0808	1010	1111	2323
	1515	1416	0909	1010	1112	1923
	1515	1416	0909	1010	1111	2323
	1414	1516	0808	1010	1112	0819
	1515	1414	0808	1010	1111	0608
	1515	1414	0909	1010	1111	0623
	1415	1416	0808	1010	1111	0623
	1515	1416	0808	1010	1212	2323
	1515	1416	0909	1010	1011	1923
	1515	1414	0808	1010	1111	0723
	1414	1416	0808	1010	1113	1923
Cam						
	1414	1414	0808	1010	1111	1919
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0623
	1414	1414	0909	1010	1011	0606
	1414	1414	0909	1010	1111	0808
	1414	1515	0808	1010	1011	0622
	1414	1416	0909	1010	1111	2222
	1414	1416	0909	1010	1111	2222
	1414	1414	0909	1010	1011	1919
	1414	1416	0909	1010	1011	0619
	1414	1414	0808	1011	1111	0623
	1414	1414	0909	1010	1111	0622
	1414	1414	0909	1010	1111	1922
	1414	1414	0909	1010	1111	0609
	1414	1416	0909	1010	1111	0606
	1414	1414	0909	1010	1111	2222
	1414	1414	0909	1010	1111	1922
	1414	1414	0909	1010	1111	0608
	1414	1414	0808	1010	1111	1919
	1414	1414	0909	1111	1111	2222
	0000	1516	0000	1010	1111	2323
	1414	1416	0808	1010	1111	0606
	1414	1414	0909	1010	1011	2323
	1414	1416	0808	1010	1111	0623
	1414	1414	0909	1010	1111	0923
	1414	1414	0909	1011	1111	0723
	1414	1416	0909	1010	1111	0623
	1414	1414	0909	1010	1011	1923
	1414	1414	0909	1010	1111	0607
	1414	1415	0909	1006	1011	0708
	1414	1414	0808	1010	1011	0606
	1414	1416	0808	1010	1111	2323
	1414	1414	0808	1011	1011	0619
	0914	1416	0808	1010	1111	0619
	1414	1415	0909	1010	1011	1923
	1414	1416	0909	1010	1111	0619
	1414	1415	0909	1010	1111	0623
	1414	1416	0909	1010	1111	0607
	1414	1416	0909	1010	1011	0608
	1414	1415	0909	1010	1111	0619
	1414	1414	0808	1010	1111	0607
	1414	1416	0808	1010	1111	0623
	1414	1414	0909	1010	1111	0823
	1414	1416	0909	1010	1111	0608
Sal						
	1414	1616	0909	1010	1111	0623

	1414	1414	0909	1010	1111	1923
	1414	1415	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0623
	1414	1414	0808	1010	1111	0606
	1414	1414	0909	1010	1111	0607
	1414	1414	0909	1010	1111	0619
	1414	1416	0909	1010	1111	0723
	1414	1414	0909	1010	1111	2323
	1414	1414	0808	1010	1111	0723
	1414	1414	0909	1010	1011	0619
	1414	1415	0909	1010	1111	0708
	1414	1414	0909	1010	1111	0606
	1414	1415	0909	1010	1111	0607
	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0919
	1414	1414	0909	1010	1111	0608
	1414	1414	0909	1010	1111	0619
	1414	1416	0909	1010	1111	0619
IseI						
	1414	1416	0909	1010	1111	0625
	1515	1416	0909	1011	1111	0619
	1515	1416	0909	1010	1111	0619
	1415	1414	0909	1010	1111	2323
	1515	1616	0909	1010	1111	0619
	1515	1416	0808	1012	1111	0606
	1414	1415	0909	1010	1111	2323
	1515	1414	0909	1010	1111	0619
	1515	1414	0909	1010	1111	0723
	1616	1416	0808	1010	1011	0623
	1515	1416	0909	1010	1111	0619
	1414	1416	0808	1010	1111	0723
	1414	1416	0808	1010	1111	2323
	1414	1416	0808	1010	1111	2310
	1414	1414	0808	1010	1111	1923
	1415	1416	0808	1010	1011	1923
	1414	1416	0909	1010	1111	0606
	1414	1414	0808	1010	1111	0606
	1111	1416	0909	1011	1111	1905
	1414	1416	0909	1010	1111	0623
	1515	1414	0909	1111	1111	1010
	1414	1414	0808	1010	1111	0619
SaI						
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1010	1923
	1414	1414	0808	1010	1111	0623
	1414	1414	0909	1010	1011	0606
	1414	1414	0909	1010	1111	0625
	1414	1415	0909	1010	1011	0623
	1414	1416	0909	1010	1111	0608
	1414	1414	0909	1010	1011	1923
	1414	1414	0909	1010	1111	1923
	1414	1416	0808	1010	1111	0623
	1414	1416	0909	1010	1111	0606
	1414	1416	0909	1010	1111	0619
	1414	1414	0909	1010	1010	0723
	1414	1414	0909	1010	1111	0919
	1414	1414	0909	1010	1111	0606
	1414	1416	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0623
	1414	1416	0909	1010	1111	0619
	1414	1414	0909	1010	1111	0723
	1414	1416	0909	1010	1111	0606
	1414	1414	0808	1010	1111	0606
	1414	1414	0909	1010	1111	0623

	1414	1414	0809	1010	1111	0607
	1414	1414	0909	1010	1111	1923
Kib						
	1515	1414	0808	1010	1011	0619
	1414	1414	0909	1010	1111	2323
	1414	1416	0909	1010	1011	1919
	1415	1414	0808	1010	1111	1905
	1414	1414	0808	1010	1111	1919
	1512	1416	0909	1010	1011	1923
	1415	1616	0808	1010	1011	0719
	1414	1515	0909	1010	1111	0719
	1414	1414	0808	1010	1111	2323
	1516	1416	0909	1010	1011	1818
	1415	1416	0808	1010	1111	0623
	1515	1416	0808	1010	1011	1919
	1414	1416	0909	1010	1111	1923
	1515	1416	0909	1010	1111	2323
	1414	1414	0808	1010	1111	2324
	1414	1414	0808	1010	1111	1923
	1515	1414	0909	1010	1011	2323
	1515	1416	0910	1010	1111	0623
	1212	1414	0808	1010	1011	0723
	1512	1414	0909	1010	1111	0723
	1415	1414	0808	1010	1111	1923
	1515	1416	0808	1010	1111	2323
	1515	1416	0808	1010	1111	1923
MalN						
	1414	1414	0909	1010	1111	0623
	1414	1415	0808	1010	1111	0723
	1414	1416	0808	1010	1111	2323
	1414	1414	0909	1010	1111	1923
	1414	1416	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0623
	1414	1416	0909	1010	1011	0719
	1414	1416	0808	1010	1011	0723
	1414	1616	0909	1010	1111	0619
	1414	1415	0808	1010	1111	0606
	1414	1416	0909	1010	1111	0619
	1414	1515	0909	1010	1111	0606
	1414	1414	0909	1111	1111	0606
	1414	1414	0909	1010	1011	0623
	1414	1415	0909	1010	1111	1923
	1414	1414	0909	1010	1111	1923
	1414	1416	0909	1010	1011	2323
	1414	1414	0909	1010	1111	0607
	1414	1414	0909	1010	1111	0624
MalW						
	1414	1415	0909	1010	1111	0623
	1414	1414	0909	1010	1111	0623
	1414	1515	0909	1010	1111	0623
	1515	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0623
	1515	1415	0909	1010	1111	0623
	1414	1414	0808	1010	1111	0623
	1414	1415	0909	1010	1111	0623
	1414	1414	0808	1010	1111	0607
	1414	1414	0808	1010	1111	0723
	1414	1414	0909	1010	1111	0723
	1414	1414	0808	1010	1111	0623
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	2323
	1414	1416	0909	1010	1111	0606
	1414	1414	0909	1010	1111	2323

	1414	1414	0808	1010	1111	2323
	1414	1613	0909	1010	1111	0623
	1414	1415	0909	1010	1111	1923
	1414	1414	0808	1010	1111	0623
	1414	1416	0909	1010	1111	0723
	1414	1414	0909	1010	1111	0723
	1415	1414	0909	1010	1111	2323
MalO						
	1414	1414	0909	1010	1111	0723
	1414	1416	0909	1010	1111	2323
	1411	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0607
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0623
	1515	1414	0808	1010	1111	0623
	1414	1414	0808	1010	1111	0623
	1414	1414	0909	1010	1111	0623
	1414	1415	0909	1010	1111	0623
	1414	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1111	0607
	1414	1415	0809	1010	1111	2323
	1414	1414	0909	1010	1111	0606
	1414	1415	0909	1010	1111	0723
	1414	1414	0909	1010	1111	1919
	1414	1414	0808	1010	1111	1923
	1414	1414	0909	1010	1111	0623
	1415	1414	0808	1010	1111	2323
	1414	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1111	0623
	1414	1414	0808	1010	1111	1923
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0607

Table A2: Genotype Matrix of *Amphitmetus transversus* containing re-estimated null alleles (‘99’). Missing genotypes indicated as ‘0000’.

	At-MS05	At-MS42	At-MS58	At-MS90	At-MS91	At-MS93
Col						
	1414	1414	0899	1010	1011	1923
	1414	1414	0899	1011	1111	0623
	1414	1414	0899	1011	1111	0707
	1414	1416	0899	1010	1111	1919
	1414	1415	0808	1010	1111	0723
	1414	1416	0808	1010	1111	0619
	1414	1516	0808	1010	1010	0606
	1414	1416	0808	1010	1111	0606
	1414	1516	0809	1010	1111	2323
	1414	1414	0809	1010	1111	0619
	1414	1415	0809	1010	1111	0606
	1414	1414	0999	1010	1111	1919
	1414	1414	0999	1010	1111	0606
	1414	1414	0999	1010	1111	0623
	1414	1515	0999	1010	1111	9999
	1414	1415	0999	1010	1111	1919
	1414	1414	0999	1010	1111	0619
	1414	1414	0999	1010	1111	1919
	1414	1414	0999	1010	1111	0606
	1414	1416	0999	1010	1111	1919
	1616	0000	0000	0000	0000	1923
	1414	1616	0999	1010	1111	1922
	1414	1414	0999	1010	1111	1919
	0000	0000	0303	1010	0000	0000

	1414	1414	9999	1010	1011	2323
	1414	1416	0999	1010	1109	0719
	1414	1416	0999	1010	1111	0619
	1414	1414	0999	1010	1111	0723
	1414	1414	0999	1010	1011	0607
	1414	1416	0909	1010	1111	1923
	1414	1416	0909	1010	1111	0606
	1414	1510	0909	1010	1111	0623
	1414	1414	0909	1010	1111	1923
	1414	1415	0909	1010	1011	1923
	1414	1414	0909	1010	1111	0623
	1414	1410	0909	1010	1111	0623
	1414	1414	0909	1010	1111	0619
	1414	1414	0909	1010	1111	1923
	1414	1415	0909	1010	1111	0619
	1414	1414	0909	1010	1011	2323
	1414	1416	0909	1010	1115	0719
	1414	1416	0909	1010	1111	0623
	1414	1414	0909	1010	1111	0619
	1414	1415	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0623
	1414	1414	0909	1010	1011	0619
	1414	1414	0909	1010	1111	0606
	1414	1415	0909	1010	1011	0619
	1414	1414	0909	1010	1111	0619
	1414	1414	0909	1010	1011	0619
BusI						
	1414	1516	0899	1010	1011	1923
	1414	1416	0899	1010	1111	0619
	1414	1415	0808	1010	1011	0623
	1414	1717	0808	1010	1111	0608
	1414	1414	0808	1010	1011	0709
	1414	1415	0999	1010	1111	0607
	1414	1415	0999	1010	1111	0607
	1414	1415	0999	1010	1011	0623
	1414	1516	0999	1010	1011	1923
	1414	1414	0999	1010	1111	0606
	1414	1416	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0619
	1415	1415	0999	1010	1011	0606
	1414	1415	0999	1010	1011	0607
	1414	1414	0999	1010	1111	1919
	1414	1415	0999	1010	1111	1923
	1414	1414	0909	1010	1111	1919
	1414	1414	0909	1010	1011	0623
	1414	1414	0909	1010	1111	1923
	1414	1416	0909	1011	1011	0623
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1011	0623
	1414	1616	0909	1010	1111	0606
	1414	1616	0909	1010	1111	0606
	1414	1415	0909	1010	1111	0619
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0619
	1414	1416	0909	1010	1111	1923
	1414	1415	0909	1010	1011	2323
	1414	1415	0909	1010	1011	0606
Kai						
	1499	1416	0808	1010	1010	1919
	1499	1414	0999	1010	1010	1923
	1499	1414	0999	1010	1010	1919
	1499	1414	0999	1010	1010	1923
	1499	1414	0909	1010	1010	1923
	1499	1414	0909	1010	1010	1923

	1414	1416	0909	1010	1010	1923
	1414	1416	0909	1010	1010	2323
	1414	1414	0909	1010	1010	2323
	1414	1414	0909	1010	1010	0619
	1414	1416	0909	1010	1011	2323
	1414	1414	0909	1010	1010	0623
	1414	1414	0909	1010	1010	1923
	1414	1416	0909	1010	1010	1919
	1414	1414	0909	1010	1010	0623
	1418	1416	0909	1010	1010	0623
	1899	1414	0909	1010	1010	1919
	1818	1414	0909	1010	1010	0606
	1818	1414	0909	1010	1010	2323
Iku						
	1499	1414	0899	1010	1099	1919
	1414	1416	0899	1010	1010	0619
	1414	1416	0899	1010	1011	0605
	1415	1415	0899	1010	1011	2323
	1415	1416	0899	1010	1011	0919
	1599	1416	0899	1010	1011	0619
	1599	1415	0899	1010	1199	2323
	1599	1516	0899	1010	1199	0623
	1599	1414	0899	1010	1199	0623
	1599	1415	0808	1010	1199	0623
	1599	1414	0808	1010	1199	1923
	1515	1416	0808	1010	1199	0605
	1515	1416	0808	1010	1111	1923
	1515	1415	0999	1010	1111	0619
	1515	1414	0999	1010	1111	0606
	1515	1414	0999	1010	1111	0606
	1515	1416	0999	1010	1111	0608
	1515	1414	0999	1010	1111	0623
	1515	1416	0999	1010	1111	0708
	1515	1416	0999	1010	1111	1924
	1515	1414	0909	1010	1111	0606
	1516	1416	0909	1010	1212	0719
	1616	1414	0909	1010	1212	1919
Buk						
	1499	1414	0899	1010	1111	2323
	1499	1414	0899	1010	1111	0719
	1499	1416	0899	1010	1011	0619
	1499	1414	0808	1010	1111	0623
	1499	1416	0808	1010	1111	0723
	1499	1412	0808	1010	1111	1923
	1499	1412	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0619
	1414	1414	0999	1010	1111	2323
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1111	1923
	1414	1414	0999	1010	1111	2323
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0624
	1414	1414	0999	1010	1111	2323
	1414	1415	0999	1010	1111	1923
	1414	1414	0999	1010	1111	0623
	1414	1410	0909	1010	1111	1923
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1111	2323
	1415	1414	0909	1010	1111	1923
	1416	1414	0909	1010	1111	0619
	1515	1416	0909	1011	1111	0723

	1616	1414	0909	1010	1111	0623
	1616	1412	0909	1010	1111	2323
IsiI						
	1414	1414	0899	1010	1111	0699
	1414	1414	0899	1010	1111	0699
	1414	1416	0899	1010	1111	0699
	1414	1416	0899	1010	1111	0699
	1414	1414	0899	1010	1111	0699
	1414	1414	0899	1010	1111	0606
	1414	1416	0808	1010	1111	0606
	1414	1416	0808	1010	1111	0606
	1414	1415	0808	1010	1111	0606
	0914	1416	0808	1010	1011	0607
	1414	1416	0999	1010	1011	0608
	0909	1414	0999	1010	1111	0608
	1414	1616	0999	1010	1111	0619
	1414	1416	0999	1010	1111	0623
	1414	1416	0999	1010	1011	0623
	1414	1416	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0624
	1414	1616	0999	1010	1010	0709
	1414	1414	0999	1010	1111	0709
	1414	1414	0999	1010	1111	0819
	1414	1414	0999	1010	1111	0909
	1414	1416	0999	1010	1111	1999
	1415	1414	0999	1010	1011	1999
	1414	1414	0909	1010	1111	1919
	1414	1414	0909	1010	1111	1923
	1414	1416	0909	1010	1111	1923
	1414	1416	0909	1010	1111	2299
	1414	1516	0909	1010	1111	2222
	1414	1414	0909	1010	1111	2222
	1414	1414	0909	1010	1011	2399
	1414	1416	0909	1010	1011	2399
	1414	1416	0909	1010	1111	2323
KiN						
	1499	1414	0899	1010	1111	1923
	1499	1416	0899	1010	1011	1923
	1499	1416	0899	1010	1012	1919
	1499	1414	0899	1010	1011	1923
	1414	1416	0899	1010	1111	1923
	1414	1416	0899	1010	1111	1923
	1414	1416	0899	1010	1111	1923
	1414	1414	0808	1010	1011	1923
	1414	1416	0808	1010	1011	1919
	1414	1414	0808	1010	1111	0623
	1414	1416	0808	1010	1111	0608
	1414	1416	0999	1010	1113	0819
	1414	1414	0999	1010	1112	1919
	1414	1414	0999	1010	1111	0606
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1011	0823
	1414	1414	0999	1010	1111	0619
	1414	1416	0999	1010	1111	2323
	1415	1414	0909	1010	1111	1923
	1415	1414	0909	1010	1111	1919
	1515	1414	0909	1010	1111	1919
	1515	1414	0909	1010	1111	1919
KiWW						
	1515	1416	0899	1011	1111	0606
	1414	1414	0808	1011	1112	1919
	1414	1413	0808	1010	1111	0623
	1414	1416	0999	1010	1111	0723

	1414	1416	0999	1010	1111	0603
	1415	1414	0999	1010	1011	0619
	1414	1414	0999	1010	1111	0505
	1414	1414	0999	1010	1012	2323
	1414	1414	0999	1010	1111	1923
	1414	1416	0909	1111	1111	1923
	1414	1416	0909	1010	1111	1923
	1415	1616	0909	1011	1111	1923
	1414	1413	0909	1011	1111	0619
	1414	1416	0909	1010	1111	0819
	1414	1414	0909	1010	1112	1919
	1414	1416	0909	1010	1111	0623
	1414	1416	0909	1010	1111	2323
KiS						
	1499	1414	0899	1010	1011	0619
	1499	1416	0899	1010	1111	0608
	1499	1416	0899	1010	1111	1919
	1499	1416	0899	1010	1109	0606
	1499	1414	9999	1010	1111	0606
	1499	1616	9999	1010	1011	0608
	1499	1414	0808	1010	1011	0622
	1499	1416	0808	1111	1010	1922
	1499	1416	0808	1010	1011	0622
	1499	1416	0808	1010	1113	0619
	1499	1414	0999	1010	1111	0623
	1499	1416	0999	1010	1111	2222
	1499	1415	0999	1011	1111	1919
	1414	1616	0999	1010	1010	0622
	1414	1414	0999	1010	1111	2323
	1414	1616	0999	1010	1011	0622
	1414	1415	0999	1010	1111	0623
	1414	1416	0999	1010	1111	2222
	1414	1416	0999	1010	1111	1919
	1414	1414	0999	1010	1113	1923
	1414	1416	0999	1010	1011	0619
	1414	1414	0999	1010	1111	1919
	1414	1416	0999	1010	1011	0606
	1414	1618	0999	1010	1111	0623
	1414	1416	0999	1010	1111	0819
	1414	1414	0999	1010	1111	0823
	1414	1414	0999	1011	1111	0619
	1414	1414	0909	1011	1111	0919
	1414	1616	0909	1010	1011	1923
	1414	1416	0909	1010	1010	1923
	1414	1416	0909	1010	1011	0623
	1414	1414	0909	1010	1112	0607
	1414	1416	0909	1011	1111	0623
	1414	1416	0909	1010	1312	2323
	1414	1416	0909	1010	1111	0608
	1414	1416	0909	1011	1111	0608
	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1012	1111	0819
	1414	1416	0909	1010	1212	2323
	1415	1414	0909	1010	1111	0623
	1415	1416	0909	1010	1012	0608
	1415	1616	0909	1010	1111	1923
	1599	1414	0909	1010	1111	1919
	1599	1414	0909	1010	1111	0605
	1515	1416	0909	1010	1111	1923
	1515	1416	0909	1012	1111	0623
	1515	1516	0909	1010	1011	2323
	1515	1415	0909	1010	1011	0823
Vih						
	1499	1416	0899	1010	1111	1923

	1499	1416	0899	1010	1111	0723
	1499	1416	0808	1010	1111	0606
	1414	1416	0808	1010	1111	2323
	1414	1616	0999	1010	1111	1923
	1599	1416	0999	1010	1111	0723
	1599	1416	0999	1010	1111	2323
	1599	1416	0999	1010	1111	1923
	1599	1414	0999	1010	1011	1818
	1599	1414	0999	1010	1111	0607
	1599	1414	0909	1010	1011	0607
	1515	1416	0909	1010	1111	0723
	1515	1416	0909	1010	1111	2323
	1515	1415	0909	1010	1111	2323
YalI						
	1499	1415	0899	1010	1111	0619
	1499	1416	0899	1010	1111	0607
	1414	1414	0899	1010	1111	2323
	1414	1417	0899	1010	1111	0623
	1415	1616	0899	1010	1011	2323
	1415	1414	0899	1010	1111	0623
	1599	1516	0899	1010	1111	1923
	1599	1414	0899	1010	1012	2323
	1599	1416	0899	1010	1111	1923
	1599	1616	0899	1010	1111	1923
	1599	1616	0808	1010	1011	0623
	1599	1416	0808	1010	1011	2323
	1599	1616	0808	1010	1112	2305
	1599	1416	0808	1010	1111	0610
	1599	1414	0808	1010	1111	1919
	1599	1616	0808	1010	1111	0623
	1515	1416	0809	1010	1111	1919
	1515	1416	0999	1010	1011	0723
	1515	1414	0999	1010	1010	0623
	1515	1416	0999	1010	1111	2323
	1515	1414	0999	1010	1111	2305
	1515	1416	0999	1010	1111	0623
	1515	1416	0999	1010	1111	0623
	1515	1416	0999	1010	1111	2323
	1515	1416	0999	1010	1112	1923
	1515	1416	0999	1010	1111	2323
	1515	1516	0999	1010	1112	0819
	1515	1414	0909	1010	1111	0608
	1515	1414	0909	1010	1111	0623
	1515	1416	0909	1010	1111	0623
	1515	1416	0909	1010	1212	2323
	1515	1416	0909	1010	1011	1923
	1515	1414	0808	1010	1111	0723
	1616	1416	0808	1010	1113	1923
Cam						
	1414	1414	0899	1010	1111	0806
	1414	1414	0899	1010	1111	0699
	1414	1414	0899	1010	1111	0699
	1414	1414	0899	1010	1011	0606
	1414	1414	0899	1010	1111	0606
	1414	1515	0899	1010	1011	0607
	1414	1416	0899	1010	1111	0607
	1414	1416	0808	1010	1111	0607
	1414	1414	0808	1010	1011	0608
	1414	1416	0808	1010	1011	0608
	1414	1414	0808	1011	1111	0609
	1414	1414	0808	1010	1111	0619
	1414	1414	0999	1010	1111	0619
	1414	1414	0999	1010	1111	0619
	1414	1416	0999	1010	1111	0619

	1414	1414	0999	1010	1111	0619
	1414	1414	0999	1010	1111	0622
	1414	1414	0999	1010	1111	0622
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1111	1111	0623
	9999	1516	9999	1010	1111	0623
	1414	1416	0999	1010	1111	0623
	1414	1414	0999	1010	1011	0623
	1414	1416	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0708
	1414	1414	0999	1011	1111	0723
	1414	1416	0999	1010	1111	0808
	1414	1414	0999	1010	1011	0823
	1414	1414	0999	1010	1111	0923
	1414	1415	0999	1006	1011	1999
	1414	1414	0999	1010	1011	1919
	1414	1416	0909	1010	1111	1919
	1414	1414	0909	1011	1011	1922
	0914	1416	0909	1010	1111	1922
	1414	1415	0909	1010	1011	1923
	1414	1416	0909	1010	1111	1923
	1414	1415	0909	1010	1111	1923
	1414	1416	0909	1010	1111	2222
	1414	1416	0909	1010	1011	2222
	1414	1415	0909	1010	1111	2222
	1414	1414	0909	1010	1111	2222
	1414	1416	0909	1010	1111	2399
	1414	1414	0909	1010	1111	2323
	1414	1416	0909	1010	1111	2323
SaI						
	1414	1616	0808	1010	1111	0623
	1414	1414	0808	1010	1111	1923
	1414	1415	0999	1010	1111	1923
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0606
	1414	1414	0999	1010	1111	0607
	1414	1414	0999	1010	1111	0619
	1414	1416	0909	1010	1111	0723
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1011	0619
	1414	1415	0909	1010	1111	0708
	1414	1414	0909	1010	1111	0606
	1414	1415	0909	1010	1111	0607
	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0919
	1414	1414	0909	1010	1111	0608
	1414	1414	0909	1010	1111	0619
	1414	1416	0909	1010	1111	0619
lseI						
	1111	1416	0899	1010	1111	0625
	1499	1416	0899	1011	1111	0619
	1499	1416	0899	1010	1111	0619
	1499	1414	0899	1010	1111	2323
	1499	1616	0899	1010	1111	0619
	1499	1416	0899	1012	1111	0606
	1499	1415	0808	1010	1111	2323
	1499	1414	0808	1010	1111	0619
	1414	1414	0808	1010	1111	0723
	1414	1416	0999	1010	1011	0623
	1414	1416	0999	1010	1111	0619
	1415	1416	0999	1010	1111	0723
	1415	1416	0999	1010	1111	2323
	1599	1416	0999	1010	1111	2310

	1599	1414	0999	1010	1111	1923
	1599	1416	0999	1010	1011	1923
	1599	1416	0999	1010	1111	0606
	1599	1414	0909	1010	1111	0606
	1599	1416	0909	1011	1111	1905
	1515	1416	0909	1010	1111	0623
	1515	1414	0909	1111	1111	1010
	1616	1414	0909	1010	1111	0619
Sal						
	1414	1414	0899	1010	1111	2323
	1414	1414	0808	1010	1010	1923
	1414	1414	0808	1010	1111	0623
	1414	1414	8090	1010	1011	0606
	1414	1414	0999	1010	1111	0625
	1414	1415	0999	1010	1011	0623
	1414	1416	0999	1010	1111	0608
	1414	1414	0999	1010	1011	1923
	1414	1414	0999	1010	1111	1923
	1414	1416	0999	1010	1111	0623
	1414	1416	0999	1010	1111	0606
	1414	1416	0909	1010	1111	0619
	1414	1414	0909	1010	1010	0723
	1414	1414	0909	1010	1111	0919
	1414	1414	0909	1010	1111	0606
	1414	1416	0909	1010	1111	0606
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	1414	1416	0909	1010	1111	0619
	1414	1414	0909	1010	1111	0723
	1414	1416	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0606
	1414	1414	0909	1010	1111	0623
	1414	1414	0909	1010	1111	0607
	1414	1414	0909	1010	1111	1923
Kib						
	1212	1414	0899	1010	1011	0619
	1215	1414	0899	1010	1111	2323
	1215	1416	0899	1010	1011	1919
	1499	1414	0899	1010	1111	1905
	1499	1414	0899	1010	1111	1919
	1499	1416	0899	1010	1011	1923
	1499	1616	0899	1010	1011	0719
	1499	1515	0899	1010	1111	0719
	1414	1414	0808	1010	1111	2323
	1414	1416	0808	1010	1011	1818
	1414	1416	0808	1010	1111	0623
	1415	1416	0808	1010	1011	1919
	1415	1416	0808	1010	1111	1923
	1415	1416	0999	1010	1111	2323
	1415	1414	0999	1010	1111	2324
	1599	1414	0999	1010	1111	1923
	1599	1414	0999	1010	1011	2323
	1599	1416	0999	1010	1111	0623
	1599	1414	0999	1010	1011	0723
	1599	1414	0909	1010	1111	0723
	1515	1414	0909	1010	1111	1923
	1515	1416	0909	1010	1111	2323
	1516	1416	9010	1010	1111	1923
MalN						
	1414	1414	0899	1010	1111	0623
	1414	1415	0899	1010	1111	0723
	1414	1416	0808	1010	1111	2323
	1414	1414	0808	1010	1111	1923
	1414	1416	0999	1010	1111	0606
	1414	1414	0999	1010	1111	0623

	1414	1416	0999	1010	1011	0719
	1414	1416	0999	1010	1011	0723
	1414	1616	0999	1010	1111	0619
	1414	1415	0999	1010	1111	0606
	1414	1416	0999	1010	1111	0619
	1414	1515	0909	1010	1111	0606
	1414	1414	0909	1111	1111	0606
	1414	1414	0909	1010	1011	0623
	1414	1415	0909	1010	1111	1923
	1414	1414	0909	1010	1111	1923
	1414	1416	0909	1010	1011	2323
	1414	1414	0909	1010	1111	0607
	1414	1414	0909	1010	1111	0624
MalW						
	1499	1415	0899	1010	1111	0623
	1499	1414	0899	1010	1111	0623
	1499	1515	0899	1010	1111	0623
	1499	1414	0808	1010	1111	0723
	1499	1414	0808	1010	1111	2323
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	1414	1415	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0623
	1414	1415	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0607
	1414	1414	0999	1010	1111	0723
	1414	1414	0999	1010	1111	0723
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1111	2323
	1414	1414	0999	1010	1111	2323
	1414	1416	0999	1010	1111	0606
	1414	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	2323
	1414	1613	0909	1010	1111	0623
	1414	1415	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0623
	1415	1416	0909	1010	1111	0723
	1515	1414	0909	1010	1111	0723
	1515	1414	0909	1010	1111	2323
MalO						
	1414	1414	0899	1010	1111	0723
	1414	1416	0899	1010	1111	2323
	1411	1414	0899	1010	1111	2323
	1414	1414	0808	1010	1111	0607
	1414	1414	0808	1010	1111	2323
	1414	1414	0809	1010	1111	0623
	1515	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0623
	1414	1415	0999	1010	1111	0623
	1414	1414	0999	1010	1111	0723
	1414	1414	0999	1010	1111	0607
	1414	1415	0999	1010	1111	2323
	1414	1414	0999	1010	1111	0606
	1414	1415	0999	1010	1111	0723
	1414	1414	0909	1010	1111	1919
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1111	0623
	1415	1414	0909	1010	1111	2323
	1414	1414	0909	1010	1111	0723
	1414	1414	0909	1010	1111	0623
	1414	1414	0909	1010	1111	1923
	1414	1414	0909	1010	1111	2323
	1414	1414	9090	1010	1111	0607

Table A3: Original Genotype matrix of *Monolepta vincta.* Missing genotypes indicated as '0000'.

	Mv-MS04	Mv-MS06	Mv-MS11	Mv-MS15	Mv-MS21	Mv-MS43	Mv-MS60	Mv-MS81	Mv-MS84
BusII									
	1313	1323	1111	1111	1415	1227	1213	1522	1111
	1313	1313	1111	1111	1417	1116	1313	1515	1111
	1213	1619	1113	1111	1214	1616	1315	1515	1212
	1213	1212	1111	1111	1516	1123	1717	1519	1212
	1213	1919	1111	1111	1214	2324	0000	1619	1212
	1313	1212	1111	1212	1314	2124	1313	2222	1111
	1313	1214	1111	1111	1416	1124	0000	0000	1212
	1313	1212	1111	1111	1415	2121	1212	2222	1117
	1112	1516	1111	1111	1516	1111	0000	1526	1111
	1113	1111	1111	1111	1414	1121	1515	1616	1112
	1313	1226	1111	1111	1414	1125	1313	2222	1112
	1313	2525	1111	1111	1214	1521	1616	1617	1212
	1213	1212	1111	1111	1213	1124	1212	2020	1212
	1113	1111	1111	1313	1214	1124	1315	2323	1111
	1111	1212	1111	1111	1114	1119	1313	1515	1212
	1112	1212	1111	1111	0000	1111	1313	1620	1212
	1313	1822	1111	1111	1520	1111	1313	2222	1212
	1313	1216	1111	1111	1414	1112	1515	1515	1212
	1213	1313	1111	1111	1415	1111	0000	2222	1212
	1313	1111	1111	1111	1416	1120	1515	1623	1212
	1213	1313	1111	1111	1115	1111	1515	2121	1111
	1313	1313	1111	1111	0000	1111	1517	1516	1212
Col									
	1313	1616	1111	1111	1316	1824	1617	1616	1112
	1313	1212	1111	1111	1414	1111	0000	1617	1212
	1113	1116	1111	1111	1417	1111	1313	1826	1212
	1313	1212	1111	1111	1314	1225	0000	1822	1212
	1313	1212	1111	1111	1417	2020	1515	1727	1212
	1313	1111	1111	1111	1515	1616	1515	1515	1212
	1212	1216	11111	1111	1516	1119	2020	2222	1112
	1313	1313	1111	1111	1215	1111	1515	1719	1212
	1313	1313	1111	1111	1418	1221	1717	1522	1212
	1313	1313	1111	1111	1415	1618	1515	1116	1112
	1212	1627	1111	1111	1215	2828	1414	0000	1111
	1313	1318	1111	1111	1315	2121	1515	2828	1212
	1313	1313	1111	1111	1315	1111	1515	1515	1111
	1213	1313	1111	1112	1414	1116	1515	1717	1111
	1213	1216	1111	1111	1316	1212	1515	1517	1216
	1313	1220	1111	1111	1316	1212	1515	1517	1111
	1313	1111	1111	1111	1417	2424	1515	1717	1112
	1213	2122	1111	1111	1314	1212	1313	1616	1112
	1313	1316	1111	1111	1315	1121	1313	2222	1212
	1113	1214	1111	1111	1617	1121	1313	1522	1111
	1213	1722	1111	1212	1417	1616	1313	1722	1111
	1212	1213	1111	1111	1414	1111	1313	1919	1414
	1313	1620	1111	1111	1414	1111	2121	1515	1212
	1313	1313	1111	1111	1414	1120	1316	1515	1212
	1213	1316	1111	1111	1314	1212	1516	1522	1111
	1313	1212	1112	1111	1314	1111	1313	1821	1212
	1113	1316	1111	1111	1315	1111	1521	2525	1212
	1313	1213	1115	1111	1417	1212	1818	1718	1212
Cam									
	1313	1313	1111	1111	1214	1112	1213	1616	1212
	1313	1313	1313	1111	1415	1112	1616	1616	1212
	1213	1313	1111	1111	1214	1216	1215	1728	1212
	1113	1621	1111	1111	1317	1121	1515	1818	1212
	1213	1616	1111	1111	1315	1212	1313	1616	1212
	1111	1212	1111	1111	1214	1121	1313	2222	1212
	1313	1616	1111	1111	1313	1212	1313	1616	1212

	1313	1212	1111	1111	1517	1212	1515	2121	1112
	1111	1717	1111	1212	1315	1121	1419	1213	1313
	1113	1313	1111	1111	1314	1121	1717	1515	1111
	1313	1416	1111	1111	1414	1212	1313	2222	1212
	1213	1325	1111	1111	1515	1616	1515	1214	1212
	1313	1919	1111	1111	1315	2124	1315	1522	1314
	1313	1313	1111	1111	1315	1115	1313	1515	1212
	1113	1313	1313	1111	1414	1616	1313	1822	1112
	1212	1111	1112	1111	1518	1313	1515	1515	1313
	1113	1222	1111	1111	1617	1223	1517	1717	1212
	1213	1818	1111	1111	1313	1414	1515	1818	1112
YalII									
	1113	1116	1111	1111	1415	1111	1515	1515	1111
	1113	1416	1111	1111	1517	1717	1315	2222	1111
	1313	1213	1111	1111	1317	2324	0000	2121	1212
	1113	2323	1111	1111	1215	1919	1313	1515	1111
	1313	1316	1111	1212	1314	1616	0000	1717	1111
	1313	1526	1111	1111	1417	1115	1515	1414	1111
	1113	1420	1111	1111	1214	2121	1313	2222	1212
	1313	2021	1111	1111	1518	1120	1515	1717	1212
	1113	1324	1111	1111	1314	1112	1515	2020	1111
	1313	1316	1111	1111	1617	1120	1313	1515	1111
	1212	1213	1111	1111	1216	1122	1313	1515	1212
	1212	1316	1111	1111	1315	1212	1315	1414	1111
	1313	1316	1111	1111	1214	1111	0000	2626	1112
	1313	1414	1111	1111	1414	1212	1515	1516	1212
	1213	1616	1111	1111	1416	1212	1616	2222	1111
	1313	1414	1111	1111	1415	1525	0000	1515	1212
	1313	1313	1414	1111	1415	1111	1313	1414	1111
	1313	1313	1111	1111	1314	1212	1321	1616	1212
	1113	1313	1111	1111	1414	1212	1315	2222	1111
	1313	1111	1111	1111	1617	1112	1313	1616	1212
	1212	1116	1111	1112	1317	1515	1315	1822	1111
	1112	1113	1114	1111	1414	1111	0000	0000	1111
IsiII									
	1313	1116	1111	1111	1415	1224	0000	1515	1112
	1213	2323	1111	1111	1314	2424	1515	1619	1212
	1313	1314	1111	1111	1317	2424	1213	1515	1212
	1213	1819	1111	1111	1515	1821	1515	1515	1111
	1313	1314	1111	1111	1516	1212	1313	1717	1212
	1313	1414	1111	1111	1213	1212	0000	2222	0000
	1313	2021	1111	1212	1214	0000	0000	2124	1111
	1313	2020	1111	1111	1215	2727	1515	1522	1111
	1313	1616	1116	1111	1313	1111	0000	1515	0000
	1313	1112	1111	1111	1314	1111	0000	1515	1112
	1213	1118	1111	1111	1314	2020	1515	1818	1414
	1213	1314	1111	1111	1314	1111	0000	1727	1212
	1213	1515	1111	1111	1315	1212	1212	1616	1212
	1113	1116	1111	1111	1414	1111	1212	1822	1111
	1313	1216	1111	1111	1417	1515	1313	1522	1313
	1313	2121	1111	1212	1313	0000	1313	1919	1111
	1313	1420	1111	1111	1215	1111	1515	2222	1516
	1313	1313	1111	1111	1113	1112	1313	2222	1111
	1213	1219	1111	1111	1415	2121	1212	1616	1111
	1113	1116	1111	1111	1314	1212	1315	2122	1212
	1313	1316	1111	1414	1618	0000	0000	0000	0000
	1313	1219	1111	1212	1417	1919	1317	2828	1414
	1113	2121	1111	1111	1314	1111	1515	2323	1212
	1313	1322	1111	1111	1718	1111	1213	1717	1212
	1313	1616	1111	1111	1414	1112	1212	1729	1111
	1113	1516	1111	1111	1415	1111	1213	1522	0000
	1212	1313	1111	1111	1212	1515	1318	1919	1111
	1313	1616	1111	1111	1616	1224	1315	1717	1212
	1313	1116	1111	1111	1314	1111	1213	1717	1717

	1213	1323	1111	1111	1214	1818	1515	2122	1212
	1113	1212	1111	1112	1417	1116	1315	1522	1212
	1313	1221	1111	1111	1414	1516	1515	1426	1212
	1313	1213	1111	1515	1518	1616	1215	2020	1111
	1313	1318	1111	1111	1315	1116	1515	2222	1111
	1213	1214	1111	1212	1517	1626	1515	1717	1212
	1112	1314	1111	1111	1315	2021	2121	2222	1111
	1313	1325	1111	1111	1414	1121	1515	1515	1616
	1111	1316	1111	1111	1414	2424	1218	2325	1414
	1313	1623	1111	1111	1416	1216	1212	1515	1111
	1113	1320	1111	1111	1415	1111	1313	2222	1112
	1313	1212	1111	0000	1517	1111	1313	0000	0000
IseI									
	1113	1616	1111	1111	1314	1112	1515	1522	1111
	1111	1212	1111	1111	1318	1116	1717	2222	1212
	1313	1212	1111	1111	1516	1112	1721	1616	1111
	1313	1116	1111	1111	1317	1121	0000	1414	1112
	1113	1414	1111	1111	1414	1212	0000	2222	1111
	1313	1325	1111	1111	1415	1112	1616	1718	1111
	1113	1321	1111	1212	1215	1616	1516	1414	1212
	1313	1321	1111	1111	1314	1621	1515	1515	1112
	1313	1213	1111	1111	1315	1616	1717	1722	1111
	1313	1315	1313	1111	1113	2121	0000	1414	1212
	1313	1416	1111	1111	1114	1111	1515	2222	1112
	1213	1919	1111	1111	1515	1121	1818	1717	1112

Table A4: Genotype Matrix of *Monolepta vincta* containing re-estimated null alleles ('99'): Missing genotypes indicated as '0000'.

	MS04	MS06	MS11	MS15	MS21	MS60	MS81	MS84	MS43
BusII									
	1313	1199	1199	1199	1415	1299	1599	1199	1227
	1313	1111	1199	1199	1417	1212	1599	1199	1116
	1213	1111	1199	1199	1214	1213	1599	1199	1616
	1213	1299	1111	1199	1516	1399	1515	1111	1123
	1213	1299	1111	1199	1214	1399	1516	1111	2324
	1313	1299	1111	1199	1314	1399	1519	1111	2124
	1313	1212	1111	1111	1416	1399	1522	1112	1124
	1313	1214	1111	1111	1415	1313	1526	1112	2121
	1112	1214	1111	1111	1516	1313	1699	1117	1111
	1113	1216	1111	1111	1414	1315	1617	1299	1121
	1313	1223	1111	1111	1414	1315	1619	1299	1125
	1313	1225	1111	1111	1214	1599	1620	1299	1521
	1213	1226	1111	1111	1213	1599	1623	1299	1124
	1113	1399	1111	1111	1214	1599	2020	1299	1124
	1111	1399	1111	1111	1114	1515	2121	1299	1119
	1112	1313	1111	1111	0000	1517	2299	1212	1111
	1313	1313	1111	1111	1520	1616	2299	1212	1111
	1313	1313	1111	1111	1414	1717	2299	1212	1112
	1213	1616	1111	1111	1415	0000	2222	1212	1111
	1313	1619	1111	1111	1416	0000	2222	1212	1120
	1213	1822	1112	1212	1115	0000	2323	1212	1111
	1313	1919	1213	1313	0000	0000	0000	1212	1111
Col									
	1313	1111	1199	1111	1316	1399	1116	1199	1199
	1313	1111	1199	1111	1414	1399	1599	1199	1199
	1113	1299	1199	1111	1417	1399	1599	1199	1199
	1313	1299	1199	1111	1314	1399	1515	1199	1199
	1313	1212	1199	1111	1417	1399	1515	1111	1199
	1313	1212	1111	1111	1515	1313	1517	1111	1111
	1212	1213	1111	1111	1516	1313	1517	1111	1111

	1313	1213	1111	1111	1215	1316	1522	1112	1111
	1313	1214	1111	1111	1418	1414	1522	1112	1116
	1313	1216	1111	1111	1415	1599	1522	1112	1119
	1212	1216	1111	1111	1215	1599	1699	1112	1120
	1313	1220	1111	1111	1315	1599	1616	1112	1121
	1313	1399	1111	1111	1315	1599	1617	1299	1121
	1213	1399	1111	1112	1414	1599	1799	1299	1299
	1213	1399	1111	1111	1316	1599	1799	1299	1299
	1313	1313	1111	1111	1316	1599	1718	1299	1299
	1313	1313	1111	1111	1417	1515	1719	1299	1212
	1213	1313	1111	1111	1314	1515	1722	1299	1212
	1313	1316	1111	1111	1315	1515	1727	1299	1221
	1113	1316	1111	1111	1617	1516	1821	1212	1225
	1213	1316	1111	1212	1417	1521	1822	1212	1699
	1212	1316	1111	1111	1414	1617	1826	1212	1699
	1313	1318	1111	1111	1414	1717	1919	1212	1618
	1313	1699	1112	1111	1414	1818	2299	1212	1824
	1213	1619	1114	1111	1314	2020	2299	1212	2020
	1313	1627	1115	1111	1314	2121	2525	1212	2199
	1113	1722	1212	1111	1315	0000	2828	1214	2424
	1313	2221	1212	1111	1417	0000	0000	1416	2828
Cam									
	1313	1111	1199	1111	1214	1213	1213	1111	1112
	1313	1299	1199	1111	1415	1215	1214	1112	1112
	1213	1299	1199	1111	1214	1399	1599	1112	1115
	1113	1222	1199	1111	1317	1399	1599	1112	1121
	1213	1399	1199	1111	1315	1399	1515	1299	1121
	1111	1399	1111	1111	1214	1399	1522	1299	1121
	1313	1399	1111	1111	1313	1313	1699	1299	1121
	1313	1399	1111	1111	1517	1313	1699	1299	1299
	1111	1313	1111	1212	1315	1315	1616	1212	1299
	1113	1313	1111	1111	1314	1419	1616	1212	1212
	1313	1325	1111	1111	1414	1599	1717	1212	1212
	1213	1416	1111	1111	1515	1599	1728	1212	1216
	1313	1699	1111	1111	1315	1599	1899	1212	1223
	1313	1699	1111	1111	1315	1515	1899	1212	1313
	1113	1621	1111	1111	1414	1515	1822	1212	1414
	1212	1717	1112	1111	1518	1517	2121	1313	1616
	1113	1818	1313	1111	1617	1616	2299	1313	1616
	1213	1919	1313	1111	1313	1717	2299	1314	2421
YalII									
	1113	1116	1111	1111	1415	1399	1499	1199	1199
	1113	1416	1111	1111	1517	1399	1499	1199	1199
	1313	1213	1111	1111	1317	1399	1414	1199	1199
	1113	2323	1111	1111	1215	1313	1599	1199	1111
	1313	1316	1111	1212	1314	1313	1599	1199	1112
	1313	1526	1111	1111	1417	1313	1599	1199	1112
	1113	1420	1111	1111	1214	1315	1599	1199	1115
	1313	2021	1111	1111	1518	1315	1515	1199	1120
	1113	1324	1111	1111	1314	1315	1516	1111	1120
	1313	1316	1111	1111	1617	1315	1699	1111	1122
	1212	1213	1111	1111	1216	1321	1699	1111	1299
	1212	1316	1111	1111	1315	1599	1799	1111	1299
	1313	1316	1111	1111	1214	1599	1799	1111	1299
	1313	1414	1111	1111	1414	1599	1822	1112	1212
	1213	1616	1111	1111	1416	1515	2020	1299	1212
	1313	1414	1111	1111	1415	1515	2121	1299	1515
	1313	1313	1414	1111	1415	1616	2299	1299	1525
	1313	1313	1111	1111	1314	0000	2299	1299	1616
	1113	1313	1111	1111	1414	0000	2299	1299	1717
	1313	1111	1111	1111	1617	0000	2299	1212	1919
	1212	1116	1111	1112	1317	0000	2626	1212	2121
	1112	1113	1114	1111	1414	0000	0000	1212	2324
IsiII									

	1313	1111	1111	1199	1415	1299	1426	1199	1199
	1213	1112	1111	1199	1314	1299	1599	1199	1199
	1313	1116	1111	1199	1317	1299	1599	1199	1199
	1213	1116	1111	1199	1515	1299	1599	1199	1199
	1313	1118	1111	1199	1516	1212	1599	1199	1199
	1313	1299	1111	1199	1213	1213	1599	1199	1199
	1313	1299	1111	1199	1214	1213	1515	1199	1199
	1313	1213	1111	1199	1215	1213	1515	1199	1111
	1313	1214	1116	1199	1313	1213	1522	1199	1111
	1313	1216	1111	1199	1314	1215	1522	1199	1111
	1213	1216	1111	1199	1314	1218	1522	1111	1111
	1213	1218	1111	1199	1314	1399	1522	1111	1112
	1213	1219	1111	1199	1315	1399	1699	1111	1112
	1113	1221	1111	1199	1414	1399	1616	1112	1116
	1313	1399	1111	1111	1417	1399	1619	1112	1116
	1313	1399	1111	1111	1313	1399	1799	1112	1121
	1313	1314	1111	1111	1215	1313	1799	1299	1299
	1313	1314	1111	1111	1113	1315	1799	1299	1299
	1213	1314	1111	1111	1415	1315	1717	1299	1299
	1113	1314	1111	1111	1314	1315	1717	1299	1299
	1313	1316	1111	1111	1618	1317	1727	1299	1216
	1313	1316	1111	1111	1417	1318	1729	1299	1224
	1113	1316	1111	1111	1314	1599	1818	1299	1224
	1313	1320	1111	1111	1718	1599	1822	1299	1599
	1313	1322	1111	1111	1414	1599	1999	1299	1515
	1113	1323	1111	1111	1415	1599	1919	1299	1516
	1212	1327	1111	1111	1212	1599	2020	1212	1699
	1313	1499	1111	1111	1616	1599	2122	1212	1626
	1313	1420	1111	1111	1314	1515	2122	1212	1818
	1213	1515	1111	1111	1214	1515	2124	1313	1821
	1113	1699	1111	1111	1417	1515	2299	1499	1919
	1313	1699	1111	1111	1414	1515	2299	1499	2020
	1313	1616	1111	1111	1518	1515	2299	1414	2021
	1313	1616	1111	1112	1315	2121	2299	1516	2199
	1213	1623	1111	1299	1517	0000	2299	1616	2499
	1112	1819	1111	1299	1315	0000	2222	1717	2499
	1313	2020	1111	1212	1414	0000	2323	0000	2424
	1111	2121	1111	1212	1414	0000	2325	0000	2727
	1313	2121	1111	1414	1416	0000	2828	0000	0000
	1113	2121	1111	1515	1415	0000	0000	0000	0000
	1313	2323	1111	0000	1517	0000	0000	0000	0000
lseI									
	1113	1299	1111	1111	1314	1599	1499	1111	1112
	1111	1212	1111	1111	1318	1599	1499	1212	1116
	1313	1212	1111	1111	1516	1515	1414	1111	1112
	1313	1315	1111	1111	1317	1516	1515	1112	1121
	1113	1316	1111	1111	1414	1616	1522	1111	1212
	1313	1320	1111	1111	1415	1799	1616	1111	1112
	1113	1321	1111	1212	1215	1717	1799	1212	1616
	1313	1325	1111	1111	1314	1718	1718	1112	1621
	1313	1414	1111	1111	1315	1821	1722	1111	1616
	1313	1416	1313	1111	1113	0000	2299	1212	2121
	1313	1616	1111	1111	1114	0000	2299	1112	1111
	1213	1919	1111	1111	1515	0000	2222	1112	1121

Table A5: Matrix of geographical distance [m] for 19 sample sites of the Kakamega Forest. Site code follows Table 2.

	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO
Col	0	3491	24962	17008	2378	2149	6145	4841	4397	11918	16403	1698	2801	13217	839	20654	11741	10281	10665
BusI	3491	0	22027	13360	3000	2787	8543	7299	6564	8667	12973	2303	802	9946	2704	17245	15206	13721	13987
Kai	24962	22027	0	13198	23440	24660	30567	28938	28383	18036	10052	24368	22695	12269	24144	5596	36975	35219	35816
Iku	17008	13360	13198	0	16142	15651	20639	19431	18707	5988	4544	15656	14137	7035	16129	7849	28229	27119	27015
Buk	2378	3000	23440	16142	0	3540	8424	7208	7537	11832	15789	2897	2929	11825	2309	19019	13594	11852	12499
IsiI	2149	2787	24660	15651	3540	0	5780	4498	3912	10102	15499	759	2112	12592	1752	19853	12778	11551	11523
KiN	6145	8543	30567	20639	8424	5780	0	1291	1989	14799	20992	6316	7819	18295	6470	25607	8208	7771	6788
KiWW	4841	7299	28938	19431	7208	4498	1291	0	742	13498	19820	5055	6514	17057	5497	26320	9255	8621	7879
KiS	4397	6564	28383	18707	7537	3912	1989	742	0	12858	2582	12788	11236	2695	13145	23534	9832	9084	8458
Vih	11918	8667	18036	5988	11832	10102	14799	13498	12858	0	7977	10448	9307	7691	11155	12646	22657	21602	21345
YalI	16403	12973	10052	4544	15789	15499	20992	19820	2582	7977	0	15212	13696	3738	15540	4677	28115	26684	26950
Cam	1698	2303	24368	15656	2897	759	6316	5055	12788	10448	15212	0	1590	12106	1134	19502	13070	11704	11760
Sal	2801	802	22695	14137	2929	2112	7819	6514	11236	9307	13696	1590	0	10480	2168	17909	14574	13250	13332
IseI	13217	9946	12269	7035	11825	12592	18295	17057	2695	7691	3738	12106	10480	0	12522	7458	25009	23468	23825
BuyI	839	2704	24144	16129	2309	1752	6470	5497	13145	11155	15540	1134	2168	12522	0	19784	12498	11021	2072
Kib	20654	17245	5596	7849	19019	19853	25607	26320	23534	12646	4677	19502	17909	7458	19784	0	32469	30866	31253
MalN	11741	15206	36975	28229	13594	12778	8208	9255	9832	22657	28115	13070	14574	25009	12498	32469	0	2262	1401
MalW	10281	13721	35219	27119	11852	11551	7771	8621	9084	21602	26684	11704	13250	23468	11021	30866	2262	0	2224
MalO	10665	13987	35816	27015	12499	11523	6788	7879	8458	21345	26950	11760	13332	23825	2072	31253	1401	2224	0

Table A6: Matrix of riverine barriers. Pair of sites separated by 0 = no river; 1= one river; 2 = two rivers. Site code follows Table 2.

	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO
Col	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
BusI	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
Kai	2	2	0	1	2	2	2	2	2	1	1	2	2	1	2	0	2	2	2
Iku	1	1	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1
Buk	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
IsiI	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
KiN	1	1	2	1	1	1	0	0	0	1	1	1	1	1	1	2	1	1	1
KiWW	1	1	2	1	1	1	0	0	0	1	1	1	1	1	1	2	1	1	1
KiS	1	1	2	1	1	1	0	0	0	1	1	1	1	1	1	2	1	1	1
Vih	1	1	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1
YalI	1	1	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1
Cam	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
Sal	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
IseI	1	1	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1
BuyI	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
Kib	2	2	0	1	2	2	2	2	2	1	1	2	2	1	2	0	2	2	2
MalN	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
MalW	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0
MalO	0	0	2	1	0	0	1	1	1	1	1	0	0	1	0	2	0	0	0

Table A7: Matrix of different vegetation types. Pair of sites in 1= the same vegetation type; 2 = different vegetation types. Site code follows Table 2.

	Col	BusI	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalI	Cam	Sal	IseI	BuyI	Kib
Col	0	1	2	1	1	1	1	1	2	2	1	1	2	1	2
BusI	1	0	2	1	1	1	1	1	2	2	1	1	2	1	2
Iku	2	2	0	2	2	2	2	2	1	1	2	2	1	2	1
Buk	1	1	2	0	1	1	1	1	2	2	1	1	2	1	2
IsiI	1	1	2	1	0	1	1	1	2	2	1	1	2	1	2
KiN	1	1	2	1	1	0	1	1	2	2	1	1	2	1	2
KiWW	1	1	2	1	1	1	0	1	2	2	1	1	2	1	2
KiS	1	1	2	1	1	1	1	0	2	2	1	1	2	1	2
Vih	2	2	1	2	2	2	2	2	0	1	2	2	1	2	1
YalI	2	2	1	2	2	2	2	2	1	0	2	2	1	2	1
Cam	1	1	2	1	1	1	1	1	2	2	0	1	2	1	2
Sal	1	1	2	1	1	1	1	1	2	2	1	0	2	1	2
IseI	2	2	1	2	2	2	2	2	1	1	2	2	0	2	1
BuyI	1	1	2	1	1	1	1	1	2	2	1	1	1	0	2
Kib	2	2	1	2	2	2	2	2	1	1	2	2	2	2	1

Table A8: Matrix of different soil structures. Pair of sites in 1= the same soil structure; 2 = different soil structures. Site code follows Table 2.

	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO
Col	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1
BusI	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1
Kai	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1
Iku	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1
Buk	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0
IsiI	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0
KiN	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
KiWW	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
KiS	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Vih	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1
YalI	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1
Cam	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1
Sal	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1
IseI	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1
BuyI	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1
Kib	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1
MalN	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0
MalW	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0
MalO	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0

Table A9: Matrix of fragmentation. Pair of sites are 1= not separated; 2 = separated by non-forested areas. Site code follows Table 2.

	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO
Col	0	1	2	1	1	1	2	2	2	1	1	1	1	1	1	1	2	2	2
BusI	1	0	2	1	1	1	2	2	2	1	1	1	1	1	1	1	2	2	2
Kai	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Iku	1	1	2	0	1	1	2	2	2	1	1	1	1	1	1	1	2	2	2
Buk	1	1	2	1	0	1	2	2	2	1	1	1	1	1	1	1	2	2	2
IsiI	1	1	2	1	1	0	2	2	2	1	1	1	1	1	1	1	2	2	2
KiN	2	2	2	2	2	2	0	1	1	2	2	2	2	2	2	2	2	2	2
KiWW	2	2	2	2	2	2	1	0	1	2	2	2	2	2	2	2	2	2	2
KiS	2	2	2	2	2	2	1	1	0	2	2	2	2	2	2	2	2	2	2
Vih	1	1	2	1	1	1	2	2	2	0	1	1	1	1	1	1	2	2	2
YalI	1	1	2	1	1	1	2	2	2	1	0	1	1	1	1	1	2	2	2
Cam	1	1	2	1	1	1	2	2	2	1	1	0	1	1	1	1	2	2	2
Sal	1	1	2	1	1	1	2	2	2	1	1	1	0	1	1	1	2	2	2
IseI	1	1	2	1	1	1	2	2	2	1	1	1	1	0	1	1	2	2	2
BuyI	1	1	2	1	1	1	2	2	2	1	1	1	1	1	0	1	2	2	2
Kib	1	1	2	1	1	1	2	2	2	1	1	1	1	1	1	0	2	2	2
MalN	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	1	1
MalW	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	0	1
MalO	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	0

Table A10: Matrix of geographical distance [m] for six sample sites of the Kakamega Forest. Site code follows Table 2.

	BusII	Col	Cam	YalII	IsiII	IseI
BusII	0	3491	2303	14215	2834	9946
Col	3491	0	1698	17500	2937	13217
Cam	2303	1698	0	16371	1476	12106
YalII	14215	17500	16371	0	16391	4690
IsiII	2834	2937	1476	16391	0	12518
IseI	9946	13217	12106	4690	12518	0

Table A11: Allele frequencies of At-MS 05. Side code follows Table 2.

Allele	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih
09						0.045				
11										
12										
14	0.825	0.983	0.660	0.142	0.732	0.939	0.864	0.882	0.709	0.245
15		0.017		0.620	0.046	0.015	0.136	0.118	0.131	0.441
16	0.017			0.053	0.076					
18			0.149							
null	0.158		0.191	0.185	0.146	0.001			0.159	0.315

Allele	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO	Total
09		0.012								0.003
11				0.031					0.021	0.003
12						0.071				0.004
14	0.120	0.988	1.000	0.346	1.000	0.356	1.000	0.786	0.917	0.710
15	0.671			0.283		0.373		0.091	0.063	0.158
16	0.024			0.031		0.018				0.012
18										0.008
null	0.185			0.309		0.182		0.122		0.103

Table A12: Allele frequencies of At-MS 42. Side code follows Table 2.

Allele	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih
10	0.021				0.018					
12					0.054					
13								0.059		
14	0.708	0.600	0.842	0.652	0.857	0.682	0.795	0.647	0.594	0.571
15	0.125	0.200		0.109	0.018	0.030			0.042	0.036
16	0.146	0.167	0.158	0.239	0.054	0.288	0.205	0.294	0.354	0.393
17		0.033								
18									0.010	

Allele	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO	Total
10										0.002
12										0.003
13								0.021		0.004
14	0.529	0.750	0.816	0.636	0.833	0.696	0.658	0.792	0.917	0.715
15	0.044	0.080	0.079	0.023	0.021	0.043	0.132	0.125	0.063	0.061
16	0.412	0.170	0.105	0.341	0.146	0.261	0.211	0.063	0.021	0.212
17	0.015									0.003
18										0.001

Table A13: Allele frequencies of At-MS 58. Side code follows Table 2.

Allele	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih
03	0.015									
08	0.146	0.130	0.053	0.379	0.160	0.213	0.333	0.137	0.135	0.203
09	0.575	0.652	0.947	0.292	0.588	0.490	0.333	0.638	0.642	0.507
10										
null	0.264	0.217		0.330	0.252	0.297	0.333	0.225	0.223	0.290

Allele	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO	Total
03										0.001
08	0.333	0.199	0.089	0.276	0.122	0.391	0.158	0.182	0.176	0.201
09	0.353	0.514	0.753	0.398	0.712	0.286	0.593	0.546	0.593	0.548
10						0.015				0.001
null	0.314	0.287	0.159	0.326	0.166	0.309	0.250	0.273	0.230	0.250

Table A14: Allele frequencies of At-MS 90. Side code follows Table 2.

Allele	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih
06										
10	0.980	0.983	1.000	1.000	0.982	1.000	1.000	0.824	0.906	1.000
11	0.020	0.017			0.018			0.176	0.073	
12									0.021	

Allele	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO	Total
06		0.011								0.001
10	1.000	0.932	1.000	0.886	1.000	1.000	0.947	1.000	1.000	0.971
11		0.057		0.091			0.053			0.027
12				0.023						0.002

Table A15: Allele frequencies of At-MS 91. Side code follows Table 2.

Allele	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih
09	0.010								0.010	
10	0.104	0.200	0.974	0.144	0.018	0.121	0.136	0.059	0.188	0.071
11	0.875	0.800	0.026	0.613	0.982	0.879	0.795	0.853	0.719	0.929
12				0.072			0.045	0.088	0.052	
13							0.023		0.031	
15	0.010									
null				0.171						

Allele	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO	Total
09										0.001
10	0.118	0.125	0.026	0.045	0.146	0.174	0.105			0.145
11	0.779	0.875	0.974	0.955	0.854	0.826	0.895	1.000	1.000	0.823
12	0.088									0.018
13	0.015									0.004
15										0.001
null										0.009

Table A16: Allele frequencies of At-MS 93. Side code follows Table 2.

Allele	Col	BusI	Kai	Iku	Buk	IsiI	KiN	KiWW	KiS	Vih
03								0.029		
05				0.043				0.059	0.010	
06	0.344	0.400	0.158	0.348	0.179	0.331	0.136	0.206	0.313	0.143
07	0.073	0.067		0.043	0.089	0.037		0.029	0.010	0.179
08		0.017		0.043		0.037	0.068	0.029	0.094	
09		0.017		0.022		0.049			0.010	
10										
18										0.071
19	0.333	0.233	0.395	0.261	0.196	0.123	0.500	0.324	0.240	0.107
22	0.010					0.074			0.094	
23	0.240	0.267	0.447	0.217	0.518	0.147	0.295	0.324	0.229	0.500
24				0.022	0.018	0.012				
25										
null						0.191				

Allele	YalI	Cam	Sal	IseI	BuyI	Kib	MalN	MalW	MalO	Total
03										0.002
05	0.029			0.023		0.022				0.010
06	0.191	0.294	0.395	0.341	0.458	0.065	0.395	0.271	0.250	0.275
07	0.044	0.053	0.132	0.045	0.063	0.087	0.105	0.125	0.146	0.070
08	0.029	0.074	0.053		0.021					0.024
09		0.021	0.026		0.021					0.009
10	0.015			0.068						0.004
18						0.043				0.006
19	0.176	0.168	0.184	0.205	0.146	0.326	0.158	0.021	0.083	0.220
22		0.126								0.016
23	0.515	0.189	0.211	0.295	0.271	0.435	0.316	0.583	0.521	0.343
24						0.022	0.026			0.005
25				0.023	0.021					0.002
null		0.076								0.014

Table A17: Allele frequencies of Mv-MS 04. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11	0.136	0.054	0.222	0.159	0.110	0.167	0.141
12	0.136	0.196	0.167	0.182	0.134	0.042	0.143
13	0.727	0.750	0.611	0.659	0.756	0.792	0.716

Table A18: Allele frequencies of Mv-MS 06. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11	0.103	0.062	0.038		0.068	0.063	0.055
12	0.240	0.215	0.094	0.400	0.114	0.126	0.198
13	0.171	0.292	0.244	0.200	0.341	0.168	0.236
14	0.034	0.015	0.019		0.136	0.084	0.048
15				0.200	0.023	0.021	0.041
16	0.069	0.154	0.113	0.200	0.182	0.168	0.147
17		0.015	0.038				0.009
18	0.017	0.015	0.038			0.031	0.017
19	0.051	0.015	0.038			0.021	0.021
20		0.015			0.023	0.042	0.013
21		0.015	0.019			0.073	0.018
22	0.017	0.015	0.019			0.011	0.010
23	0.017				0.045	0.042	0.017
24					0.045		0.008
25	0.017		0.019				0.006
26	0.017				0.023		0.007
27		0.015				0.011	0.004
null	0.247	0.154	0.324			0.140	0.144

Table A19: Allele frequencies of Mv-MS 11. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11	0.863	0.790	0.730	0.932	0.988	0.958	0.877
12	0.042	0.081	0.024				0.024
13	0.021		0.094			0.042	0.026
14		0.016		0.068			0.014
15		0.016					0.003
16					0.012		0.002
null	0.074	0.097	0.152				0.054

Table A20: Allele frequencies of Mv-MS 15. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11	0.777	0.946	0.944	0.932	0.668	0.917	0.864
12	0.039	0.054	0.056	0.068	0.090	0.083	0.065
13	0.039						0.006
14					0.020		0.003
15					0.020		0.003
null	0.145				0.202		0.058

Table A21: Allele frequencies of Mv-MS 21. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11	0.050				0.012	0.083	0.024
12	0.125	0.036	0.083	0.091	0.085	0.042	0.077
13	0.050	0.196	0.278	0.136	0.207	0.250	0.186
14	0.450	0.375	0.250	0.364	0.329	0.250	0.336
15	0.175	0.179	0.250	0.159	0.183	0.250	0.199
16	0.100	0.089	0.028	0.091	0.061	0.042	0.069
17	0.025	0.107	0.083	0.136	0.085	0.042	0.080
18		0.018	0.028	0.023	0.037	0.042	0.025
20	0.025						0.004

Table A22: Allele frequencies of Mv-MS 43. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11	0.500	0.278	0.168	0.241	0.258	0.333	0.296
12	0.045	0.159	0.287	0.206	0.124	0.208	0.172
13			0.048				0.008
14			0.048				0.008
15	0.023		0.024	0.069	0.048		0.027
16	0.068	0.079	0.120	0.034	0.067	0.250	0.103
17				0.034			0.006
18		0.026			0.029		0.009
19	0.023	0.013		0.034	0.019		0.015
20	0.023	0.040		0.034	0.029		0.021
21	0.114	0.066	0.120	0.034	0.048	0.208	0.098
22				0.017			0.003
23	0.045		0.024	0.017			0.014
24	0.114	0.040	0.024	0.017	0.077		0.045
25	0.023	0.013		0.017			0.009
26					0.010		0.002
27	0.023				0.019		0.007
28		0.026					0.004
null		0.260	0.138	0.244	0.274		0.152

Table A23: Allele frequencies of Mv-MS 60. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
12	0.100		0.042		0.183		0.054
13	0.299	0.193	0.292	0.411	0.240		0.239
14		0.026	0.021				0.008
15	0.219	0.283	0.271	0.338	0.298	0.275	0.281
16	0.040	0.039	0.042	0.048		0.118	0.048
17	0.060	0.039	0.063		0.011	0.196	0.061
18		0.026			0.023	0.079	0.021
19			0.021				0.003
20		0.026					0.004
21		0.039		0.024	0.023	0.039	0.021
null	0.283	0.330	0.250	0.179	0.222	0.293	0.259

Table A24: Allele frequencies of Mv-MS 81. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11		0.015					0.003
12			0.038				0.006
13			0.019				0.003
14			0.019	0.085	0.009	0.176	0.048
15	0.219	0.198	0.134	0.156	0.166	0.088	0.160
16	0.127	0.091	0.154	0.071	0.046	0.059	0.091
17	0.018	0.167	0.058	0.057	0.111	0.117	0.088
18		0.061	0.096	0.014	0.028	0.029	0.038
19	0.036	0.046			0.046		0.021
20	0.055			0.028	0.019		0.017
21	0.036	0.015	0.038	0.028	0.028		0.024
22	0.200	0.137	0.115	0.128	0.176	0.235	0.165
23	0.055				0.028		0.014
24					0.009		0.002
25		0.030			0.009		0.007
26	0.018	0.015		0.028	0.009		0.012
27		0.015			0.009		0.004
28		0.030	0.019		0.019		0.011
29					0.009		0.002
30							
null	0.235	0.178	0.309	0.403	0.279	0.296	0.283

Table A25: Allele frequencies of Mv-MS 84. Side code follows Table 2.

Allele	Bus	Col	Cam	YalII	IsiII	IseI	Total
11	0.262	0.272	0.115	0.435	0.269	0.583	0.323
12	0.489	0.487	0.574	0.274	0.269	0.417	0.418
13			0.115		0.019		0.022
14		0.029	0.023		0.056		0.018
15					0.009		0.002
16		0.014			0.028		0.007
17	0.018				0.019		0.006
null	0.231	0.198	0.173	0.291	0.333		0.204

Table A26: UTM-Coordinates of the sample sites at the Kakamega Forest based on Arc 1960.

Sample Site	Site Code	X-Coord. (UTM)	Y-Coord. (UTM)
Colobus	Col	707028	39657
Busumbuli II	BusII	707937	35607
Busumbuli I	BusI	707951	36609
Salazar	Sal	708106	37139
Campsite	Cam	707929	39086
Isiukhu I	IsiI	708769	39253
Isiukhu II	IsiII	709289	39081
Buyangu I	BuyI	707067	39023
Buyangu II	BuyII	707042	38203
Bukhyawa	Buk	705389	38242
Shiamololi	Shi	707512	31436
Yala I	YalI	709504	23324
Yala II	YalII	709355	22381
Ikuywa	Iku	713916	24157
Isecheno I	IseI	707329	26473
Isecheno II	IseII	707329	26473
Center	Cen	709219	30390
Plantations	Pla	710014	26024
Vihiga	Vih	714223	30237
Kibiri	Kib	708030	19337
Bunyala	Bun	690312	42361
Malawa West (Malawa)	MalW	704865	50128
Malawa East (Shitirira)	MalE	707163	50237
Malawa North (Mungaha)	MalN	706311	51801
Kisere North	KiN	710704	44881
Kisere South	KiS	710435	42566
Kisere Center	KiWW	710373	43521
Kisere Fogging	KiF	710819	43113
Kisere East	KiE	711640	44860
Kaimosi	Kai	704994	14656

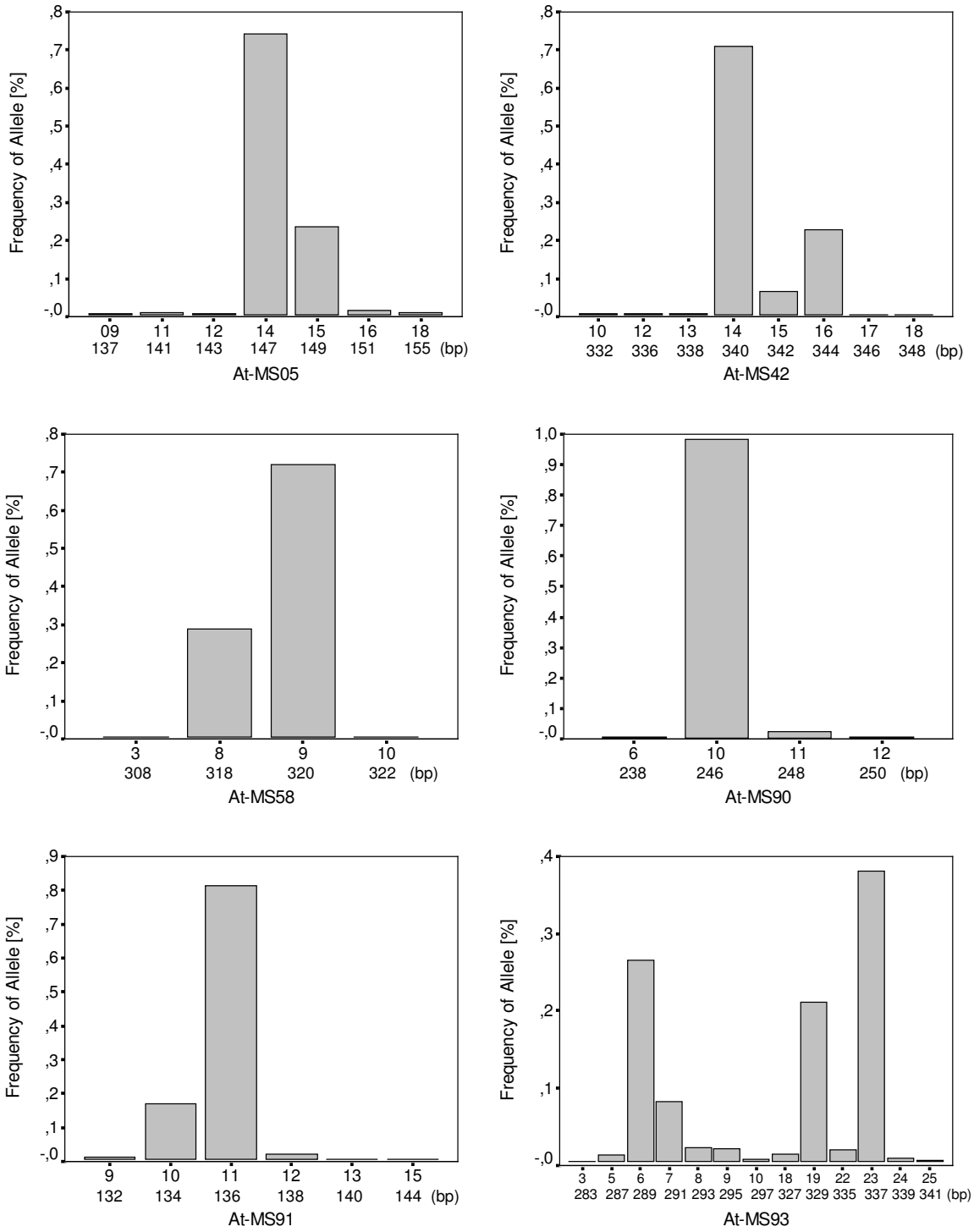
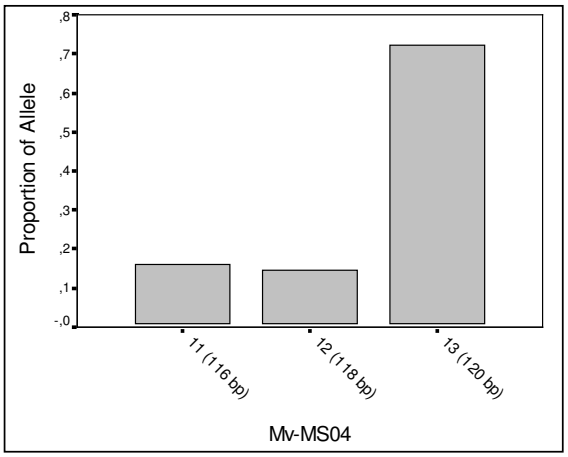
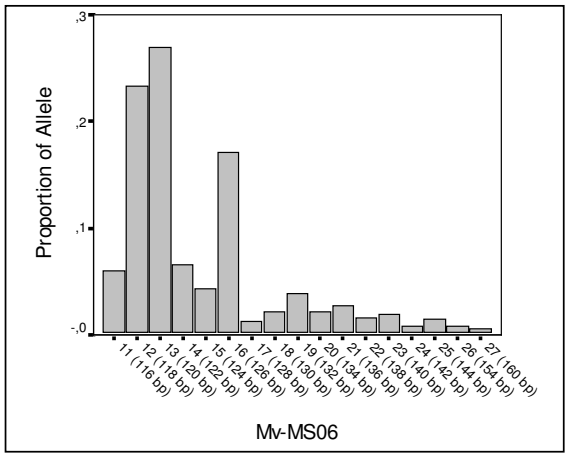
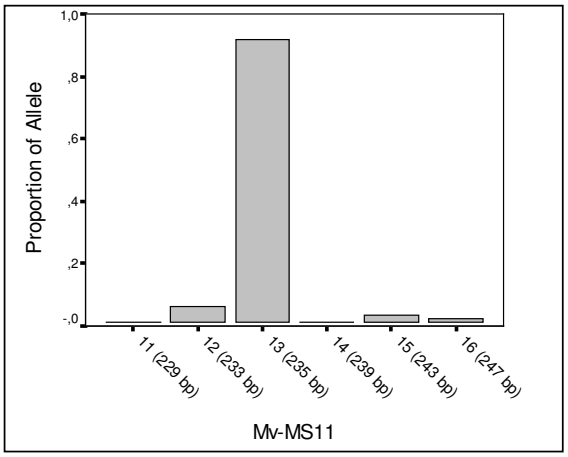
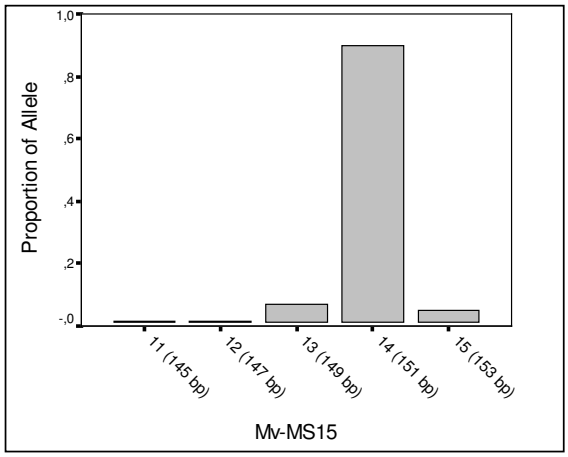


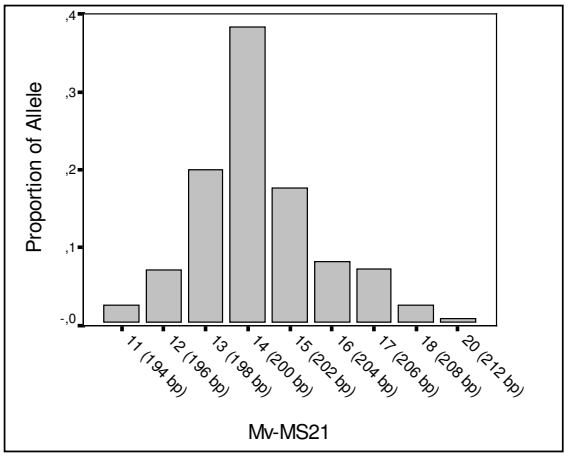
Fig. A1: Allele frequencies per loci for six microsatellite markers of *Amphitmetus transversus*. Allele identity as well as allele sizes in base pair (bp) are given.

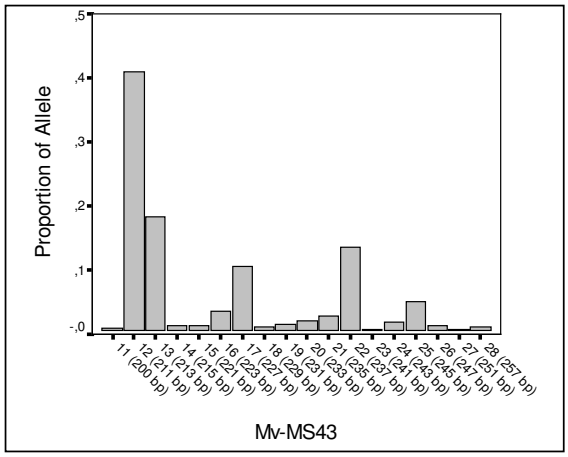

Proportion of Allele
,8
,7
,6
,5
,4
,3
,2
,1
-,0
11 (116 bp)
12 (118 bp)
13 (120 bp)
Mv-MS04


Proportion of Allele
,3
,2
,1
-,0
11 (116 bp)
12 (118 bp)
13 (120 bp)
14 (122 bp)
15 (124 bp)
16 (126 bp)
17 (128 bp)
18 (130 bp)
19 (132 bp)
20 (134 bp)
21 (136 bp)
22 (138 bp)
23 (140 bp)
24 (142 bp)
25 (144 bp)
26 (154 bp)
27 (160 bp)
Mv-MS06


Proportion of Allele
1,0
,8
,6
,4
,2
-,0
11 (229 bp)
12 (233 bp)
13 (235 bp)
14 (239 bp)
15 (243 bp)
16 (247 bp)
Mv-MS11


Proportion of Allele
1,0
,8
,6
,4
,2
-,0
11 (145 bp)
12 (147 bp)
13 (149 bp)
14 (151 bp)
15 (153 bp)
Mv-MS15


Proportion of Allele
,4
,3
,2
,1
-,0
11 (194 bp)
12 (196 bp)
13 (198 bp)
14 (200 bp)
15 (202 bp)
16 (204 bp)
17 (206 bp)
18 (208 bp)
20 (212 bp)
Mv-MS21


Proportion of Allele
,5
,4
,3
,2
,1
,0
11 (200 bp)
12 (211 bp)
13 (213 bp)
14 (215 bp)
15 (221 bp)
16 (223 bp)
17 (227 bp)
18 (229 bp)
19 (231 bp)
20 (233 bp)
21 (235 bp)
22 (237 bp)
23 (241 bp)
24 (243 bp)
25 (245 bp)
26 (247 bp)
27 (251 bp)
28 (257 bp)
Mv-MS43

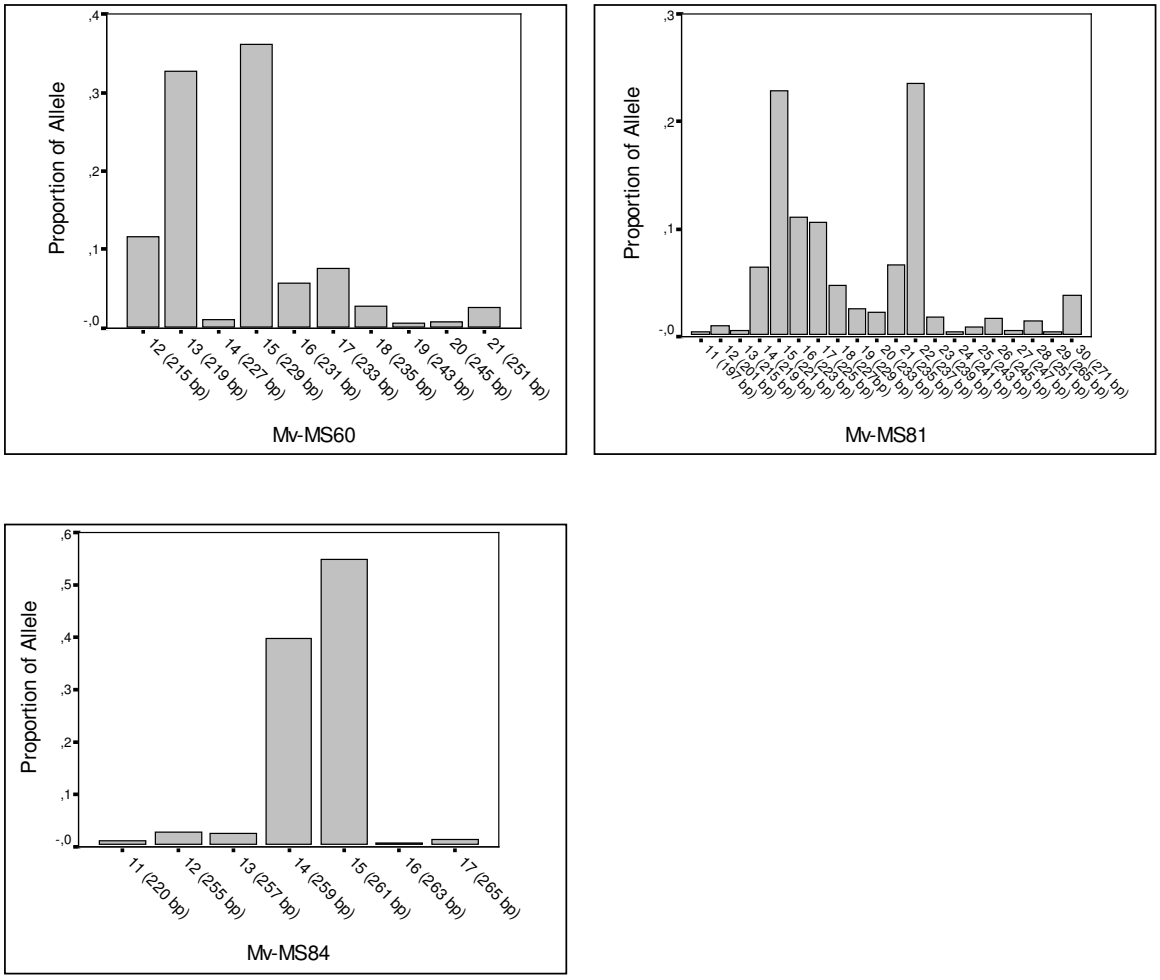


Fig. A2: Allele frequencies per loci for nine microsatellite markers of *Monolepta vincta*. Allele identity as well as allele sizes in base pair (bp) are given.

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Erklärung:

Ich versichere, dass ich die vorliegende Arbeit selbständig verfasst und ausschließlich die angegebenen Quellen und Hilfsmittel benutzt habe.

Bonn, den (Alexandra Patt)