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Repeated sampling detects gene flow in a flightless ground beetle in a fragmented landscape

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Secondary clines level down in the course of time if the gene flow is not interrupted. Temporally repeated sampling of populations in a cline allows the investigation not only of its occurrence but also of the estimation of the amount of ongoing gene flow. We reinvestigated an allozyme gradient in *Carabus auronitens* populations in the Westphalian Lowlands (northwestern Germany) 15 to 20 years after it had originally been recorded. A total of 977 individuals of this flightless woodland species from 29 sample sites were genotyped at the diallelic Est-1 locus in 2005–2006 and compared to former findings, collected in 1985–1994 from the same populations. Both data sets showed clinal variation. Pairwise differences between the samples of both data sets indicated significant decrease in the steepness of the cline during the past 15 to 20 years. The estimated average gene flow per generation is 0.6% of each beetle population. Ongoing gene flow in the flightless ground beetle *C. auronitens* led to a less pronounced cline despite a stable degree of fragmentation (and connectivity) of the landscape. Migration and gene flow were obviously enabled by the numerous hedgerows. The corridors are seen to be a prerequisite for migration between populations and for possible future range shifts of forest insect species.

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Changes in land-use influence occurrence and distribution of biota are among the most important factors affecting biodiversity in terrestrial ecosystems (Tylianakis et al. 2008). In order to maintain biodiversity, the patterns of future reactions to these changes need to be understood for as many species as possible. Analyses of the genetic structure of a species' populations allow us to estimate the impacts of anthropogenic landscape modifications (Johansson et al. 2005; Sander et al. 2006) such as habitat fragmentation (reviewed by Keyghobadi 2007; Holderegger and Di Giulio 2010) and habitat defragmentation (Hale et al. 2001; Drees et al. 2008).

One means of assessing how populations of a given species react to a given land-use pattern is a re-investigation of the respective populations after a time lag. However, repeated studies of the same populations are rare in statistical genetics (Wang and Whitlock 2003) and have mostly only been used to estimate the effective population size (Jorde and Ryman 1996; Waples and Yokota 2007; Chevolot et al. 2008). Temporally repeated sampling, however, also makes it possible to investigate recent bottlenecks in a population (Luikart et al. 1999), to study long-term effects of habitat fragmentation (Habel et al. 2011), or to estimate the migration rate (Wang and Whitlock 2003; Kim et al. 2009).

Whilst the latter method calculates the migration rate of equilibrium populations uninfluenced by selection or other non-random processes (Wang and Whitlock 2003), systems in which the genetic changes are predictable can also be used to estimate the migration rate. Such systems are secondary clines (Endler 1977) which have developed from two (or more) differentiated populations which admix and thus shape a gradient in allele frequencies in the admixed populations. Examples of such clines are the well-known hybrid zones in central Europe (reviewed by Hewitt 1999) or North America (Adams et al. 2006), and smaller scale gradients in various regions of the world (Pohl 1998; Hale et al. 2001; Drees et al. 2008). Assuming there is no selective disadvantage of heterozygotes, ongoing gene flow should level high initial differences to uniform distribution of the different alleles at the investigated loci. Although differences in neutral markers disappear quickly in contact zones, as shown in simulation studies (Durrett et al. 2000), the time required for a secondary cline to level can be used to estimate the effective gene flow of the respective population sources.

The flightless woodland ground beetle *Carabus auronitens* Fabricius 1792 shows a striking genetic cline at one allozyme locus (Terlutter 1990; Niehues et al. 1996) in an area of the Westphalian Lowlands (northwestern Germany) which was shown to be a result of fragmentation and defragmentation events (Terlutter 1990; Niehues et al. 1996; Drees et al. 2008). Fragmentation

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occurred during Middle Ages and early modern times (until about 1800) when woodlands were restricted to small remnants in an intensively used landscape. Both the implementation of modern forestry and numerous directives to establish hedgerows led to the development of a landscape with numerous forests connected by hedgerows, i.e. a defragmented landscape. Forests and hedgerows increased in area and in length, respectively, during the 19th and the first part of the 20th century (up to 1930) (Kraft and Rieger 1993). Despite some land consolidation during the 20th century the general landscape setting has been preserved until this day. This pattern of landscape development is unique in (central) Europe as in other regions the areas covered by forests are still decreasing (Desender 2005).

In the contemporary landscape of the Westphalian Lowlands, there are connecting elements, such as hedgerows, as potential corridors as well as separating elements (e.g. highways, railroads and roads). This raises the question about the relative importance of the connecting and separating elements of the landscape. If connecting elements have allowed significant gene flow to take place, then further gene flow is expected to reduce the amount of genetic differentiation within the region of the cline and to change the shape of the gradient, making it more levelled. We hypothesise that gene flow between the populations would lead to specific changes that are not uniform for the whole data set: Populations in the part of the cline with low s-allele frequencies (in the old data set) were expected to show an increase of s-alleles while populations at the opposite end of the cline (with high s-allele frequencies in the old data set) would decrease in s-allele proportions. The populations in the centre of the cline are expected to change least. We address this hypothesis by means of a re-investigation of 29 populations from the region of the cline 12 to 20 years after they were first sampled.

MATERIAL AND METHODS

Study species and study area

Carabus auronitens is a flightless ground beetle which mainly inhabits forests (Turin et al. 2003). For more than two decades this species has been the subject of detailed studies of its ecology, population biology, evolutionary genetics and dispersal ability (Nève and Baguette 1990; Terlutter 1990; Niehues et al. 1996; Weber and Heimbach 2001; Desender et al. 2002; Drees et al. 2008). The population sizes of *C. auronitens* tended to be stable: The results of long-term-studies showed only three-fold (in our study area, Weber and Heimbach 2001) or up to nine-fold temporal fluctuations in population size (Lüneburger Heide (northern Germany), Günther and Assmann 2004), both low values as compared to other carabid beetles.

The study area is located in the Westphalian Lowlands (northwestern Germany) southwest of Münster (Fig. 1) and spans the whole range of the cline over which the frequency of the allele EST-1 varies by 90% (Niehues et al. 1996). The area is a fragmented mosaic-like landscape in which forests and numerous hedgerows are embedded in a system of arable fields and meadows. Moreover, a system of highways, roads and railroads exists together with mostly small settlements. During the last decades, i.e. within the timeframe of our study, the landscape did not change significantly: the today's mean wooded area (16.6%) changed within the last 20 years less than 1%, as shown by a comparison of contemporary maps (Landesvermessungsamt Nordrhein-Westfalen, 1993, 2008). A similar situation has been found for a neighbouring region (Bangert and Kowarik 2000). Both, hedgerows and forests are protected by law (Naturschutz- und Waldgesetz) so that only few changes have been allowed.

Data sets

A total of 977 individuals (501 males and 476 females) were sampled from 29 sample sites distributed over the entire extent of the studied cline (henceforth referred to as 'new data set') between November 2005 and March 2006. These sites had already been investigated between 1985 and 1987 (Terlutter 1990) and between 1989 and 1994 (Niehues et al. 1996), respectively (henceforth referred to as 'old data set'). Sampling sites as well as sampling sizes and allele frequencies from the old data set (1082 individuals) are listed in Appendix 1 Table A1.

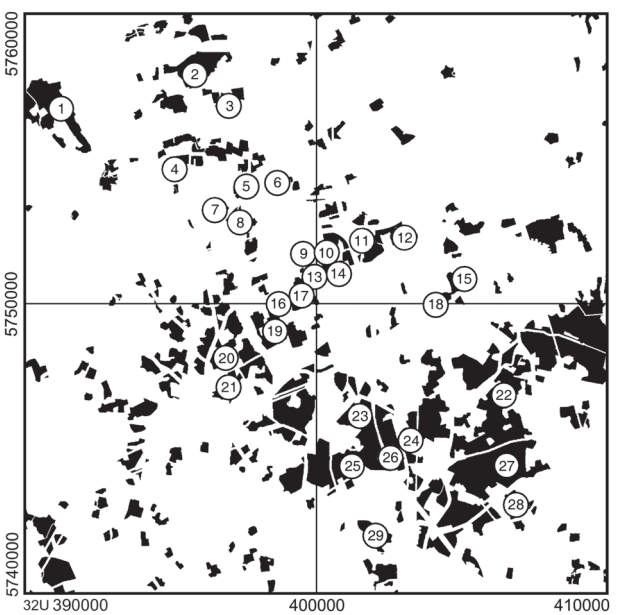

Fig. 1. Study area with 29 study sites in a mosaic of forest (black) and surroundings (arable fields, streets and settlements). Numbers indicate UTM coordinates.

Sampling and electrophoresis

In 2005–2006, a mean number of 34 individuals per sampling site was collected from the beetles' wintering grounds (Appendix 1 Table A1). Each living individual we captured was sexed and 5 µl of its haemolymph was drawn by means of a sharpened capillary. The investigated animals were kept in tanks with soil at 4°C and were released at their places of origin in the subsequent spring.

The haemolymph was frozen and stored at –20°C (in 60 µl of a 0.15 M Tris-citrate buffer, pH 9.0, 30% saccharose) until electrophoresis. The haemolymph esterase (EST-1) was analysed using the electrophoretic methods for acrylamide gels (5.5%) and run in a Tris-borate buffer (pH 9.0, cf. Assmann et al. 1994). The haemolymph esterase was stained according to Richardson et al. (1986).

Tests on Hardy-Weinberg-equilibrium and F_{ST} -calculations

As the investigated haemolymph esterase locus is X-linked in *C. auronitens* (Terlutter 1990), the females (the homogametic sex in *Carabus*, Weber 1966) of each population were tested for deviation from Hardy–Weinberg-equilibrium (HWE exact test, exact test using a Markov chain, Arlequin ver. 3.11, Excoffier et al. 2005). Allele frequencies of males and females were tested for deviation from uniformity (Fisher's exact test, implemented in 'Rcmdr' package, Fox 2005, using R 2.8.1, R Development Core Team 2009).

Pairwise temporal F_{ST} values ($F_{ST\ obs}$) were calculated for each sample site from the allele frequencies (thus also taking the males into account) following Wright (1978). Mean pairwise F_{ST} values (after Wright) per time frame were taken from spatial autocorrelation analyses.

Analyses of the clines

The occurrence of clinal variation was investigated using spatial autocorrelation analysis (cf. Manel et al. 2003) by means of Spatial Genetic Software (SGS, ver. 1d, Degen et al. 2001), which enabled us to study spatial autocorrelation from allele frequencies alone. Nine distance classes (2 km each) were considered in this study. Significant deviations from a spatially random distribution were calculated using a Monte Carlo permutation (of 1000 permutations) implemented in SGS. A set of pairwise F_{ST} values increasing from significantly negative to significantly positive scores describes a cline (Barbujani 2000), as has already been shown for microsatellite data from this area (Drees et al. 2008).

Multiple linear regression analyses describe the direction of the cline: The relationship between dependent (allele frequency, arcsin-transformed) and independent variables (locality of the sample sites: longitude (Xgeo) and latitude (Ygeo)) is described mathematically by the equation of a regression plane, which indicates a clinal change of the allele frequency in space. The direction of the plane slope is referred to as the direction of the gradient in which the allele frequency proportions change most markedly (cf. Drees et al. 2008).

In order to simplify the clines to two-dimensional-clines the sites were projected orthogonally onto a line following the direction of the gradient (position in the cline), and a simple linear regression analysis was performed to describe the cline. Regression analyses were conducted in R (ver. 2.8.1, R Development Core Team 2009), other calculations were performed in Excel (ver. 2007).

Analyses of the potential shift in allele frequencies

To test changes of allele frequencies across the cline we applied a repeated-measure one-way ANOVA with samples grouped according to their position in the cline: northwest (0–6.2 km, populations 1–8), centre (6.2–12.4 km, populations 9–21), and southeast (12.4–18.6 km, populations 22–29) (factor cline). The repeated sampling is described by the factor time. Post-hoc tests were performed as a series of paired t-tests for the population groups, corrected for multiple testing (Benjamini et al. 2001). Calculations were carried out in R. The mean allele frequencies of both data sets were compared using the Wilcoxon test for pairwise differences (implemented in 'Rcmdr' package, Fox 2005).

In order to analyse the influence of landscape features, such as distance to nearest patch and patch size we checked the differences in the s-allele frequency in time (figures of new data set minus figures of old data set, both arcsin-transformed) at each site directly by means of stepwise multiple linear regression analysis (in R). The position in the cline acted as the explanatory variable, as did patch size and distance (i.e. linear distance to nearest patch).

Estimation of the dimension of the migration rate

We estimated the number of migrants by using a simplified stepping stone model. Ten sample sites (randomly chosen from the 29 sites) were arranged according to their geographical position along the cline. The number of individuals at the chosen sites was calculated from the size of the respective site multiplied by the long-term mean density of *C. auronitens* in this region (Weber and Heimbach 2001).

As a measure of temporal genetic changes, temporal pairwise F_{ST} values ($F_{ST\ obs}$, F_{ST} calculated according to Wright 1978) were calculated from each site's allele frequencies taken from both data sets.

For the simulation of the migration rate, lower migration rates were subsequently tested until simulated pairwise F_{ST} values fit observed temporal genetic changes. Initial allele frequencies of haemolymph esterase (generation 0) were

taken from the old data set. In each generation an unknown proportion of migrating beetles (*m*) disperses. Initially, a migration rate of 0.1 was used, reflecting twice the migration rate of marked individuals estimated from capture–mark–recapture-experiments (cf. Drees 2003) and, thus, considered the maximum value which *m* can reach. In the one-dimensional stepping stone model, the beetles are only able to reach the two neighbouring forests with an equal migration rate between each of the neighbouring forests, irrespective of the distance between them. Allele frequencies in the following generation were re-calculated taking the immigrating and emigrating individuals into consideration, while assuming the population size to be stable. After 15 generations with a constant migration rate *m* between each of the 10 sites, the temporal pairwise F_{ST} values between the allele frequencies at the beginning of the simulation (old data set) and the simulated frequencies were calculated according to Wright (1978). These pairwise F_{ST} values ($F_{ST\ sim}$) were compared with those from the observations ($F_{ST\ obs}$) by calculating the difference between them ($\Delta\Delta F_{ST}$). The procedure was repeated with a lower *m* value (–0.0001 in each step) until the $\Delta\Delta F_{ST}$ over the 10 sites was at its minimum. This lower *m* value is referred to as the estimated migration rate. The entire procedure was repeated 50 times with 10 randomly chosen sites, respectively, and the resulting migration rate was averaged.

RESULTS

Clinal variation

For the old data set (1985–1994) the allele frequencies of the s-allele in *Carabus auronitens* from 29 sites revealed a clinal variation in which the frequency of the s-allele increased regularly from SSE to NNW (329°, Table 1, Fig. 2, left). Spatial autocorrelation analysis revealed a typical clinal pattern with lower F_{ST} values for population pairs from small distance classes and significantly larger F_{ST} values for population pairs from large distance classes (Fig. 3). The s-allele frequencies are significantly determined by their position in the cline (Table 2) and also, for the old data set, by patch size. The simplified model, however, does not show this relationship anymore which indicates the clinal distribution of the allele frequencies to be the more important factor.

In the new data set (2005–2006), frequencies of the s-allele ranged from 0.032 (population 28) to 1.000 (population 1) with a mean of 0.493 (Appendix 1 Table A1, Fig. 2, right). Spatial autocorrelation analysis revealed clinal variation (Fig. 3), as did linear regression analysis. The frequency of the s-allele was found to increase regularly from SSE to NNW (319°, Table 1, Fig. 2). The s-allele frequencies of this data set are not significantly influenced by patch size or distance between the patches but are determined only by their position in the cline (Table 2).

Comparison of old and new data sets

The populations display different changes in s-allele frequency according to their position on the cline. The one-way repeated measure ANOVA revealed a significant effect for the interaction term time × cline ($F = 8.704$, $df = 2, 26$, $p = 0.001$). Therefore, s-allele frequencies are changing over time and, moreover, in different ways in different parts of the study area. While in the populations in the northwestern part of the cline the s-allele frequencies generally decreased over time, the populations in the southeastern part showed an increase in s-allele-frequencies (Fig. 4).

The overall F_{ST} values generally decreased but revealed no significant result ($F_{ST\ old} = 0.432$, $F_{ST\ new} = 0.348$, using Wright's estimations).

The observed changes in the cline over time are not influenced by landscape features, such as patch size or distance between the patches (Table 2). As for the observed allele frequencies, the differences between the allele frequencies of the old and new data set are only dependent on the position in the cline (Table 2).

Mean s-allele frequency does not change significantly between the old (s-allele frequency = 0.515) and new data set (0.492) (Wilcoxon test for pairwise differences, $p = 0.25$). Moreover, significant deviation from Hardy-Weinberg equilibrium was not detected for any of the sampled populations.

Table 1. *Multiple linear regression analyses: frequency of s-allele (arcsin-transformed) of the old and new data set as related to geographical coordinates. The direction of the cline (also given) was calculated from the significant regression plane.*

Data set	Explanatory variables	Estimate	p (coefficients)	R^2	p (model)	Direction of the cline
Old data set (n = 29)	Intercept	-2.161×10^{4}	2.99×10^{-7}	0.8896	3.62×10^{-13}	329°
	Xgeo	-2.397×10^{-3}	0.0006			
	Ygeo	3.932×10^{-3}	5.14×10^{-8}			
New data set (n = 29)	Intercept	-1.573×10^{4}	6.26×10^{-6}	0.8819	8.71×10^{-13}	319°
	Xgeo	-2.539×10^{-3}	6.39×10^{-5}			
	Ygeo	2.920×10^{-3}	9.74×10^{-7}			

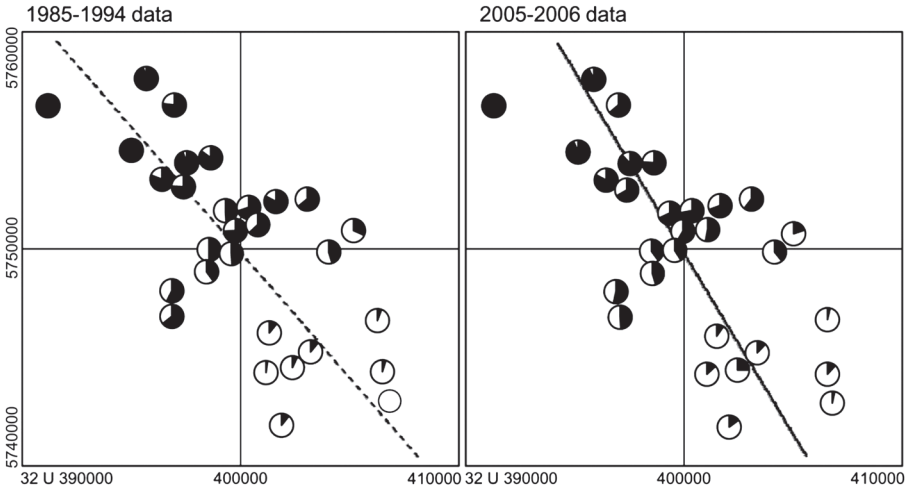

Fig. 2. Comparison of old and new data set: allelic frequencies of the s- (black) and f- (white) allele of the haemolymph esterase locus of the *Carabus auronitens* populations studied. The directions of the clines are indicated (compare Table 1).

Estimation of gene flow

Pairwise temporal F_{ST} values ($F_{ST\ obs}$) range from 0.000 (population 1) to 0.060 (population 26) with a mean of 0.018 (Appendix 1 Table A1). Between 2.4 and 62 individuals out of 1000 per generation (mean effective migration rate) were estimated to have successfully moved from one population to a neighbouring one in order to reach similar values of differentiation and thus triggered the change in allele frequencies over the 15 years. The estimated effective average migration rate of 0.61% individuals per generation (standard error: 0.169) led to the observed significant levelling of the cline.

DISCUSSION

Significant levelling of the cline – evidence for recent gene flow

Our study is one of the first investigations of the temporal changes in the genetic structure of natural populations of terrestrial animals (Hale et al. 2001; Johnson et al. 2004; Jehle et al. 2005; Yanchukov et al. 2006; Habel et al. 2011), whereas studies on marine or limnic organisms have frequently been conducted (Jorde and Ryman 1996; Chevolot et al. 2008; Demandt 2010). Despite the short

Table 2. *Multiple linear regression analyses of (1) s-allele frequencies (arcsin-transformed) of the old data set, (2) s-allele frequencies (arcsin-transformed) of the new data set and (3) the differences between old and new data set as related to the position in the cline, patch size of the patch and distance to the nearest patch. Differences in s-allele frequencies between old and new data set are the s-allele frequencies of samples in old data set minus s-allele frequencies of the samples from the same sample sites in new data set. R^2 and p values are given for the final models after model simplification.*

Response variable	Explanatory variables in the original model	p (original model)	Explanatory variable in the final (reduced) model	R^2 (final model)	p (final model)
(1) Frequency of s-allele (Old data set)	Position in the cline (329°)	1×10^{-11}	Position in the cline (329°)	0.8857	1.9×10^{-14}
	Patch size	0.027	–		
	Distance to nearest patch	0.100	–		
(2) Frequency of s-allele (New data set)	Position in the cline (319°)	6×10^{-11}	Position in the cline (319°)	0.8776	4.8×10^{-14}
	Patch size	0.283	–		
	Distance to nearest patch	0.350	–		
(3) Difference in s-allele frequency	Position in the cline (329°)	0.094	Position in the cline (329°)	0.2322	0.004
	Patch size	0.173	–		
	Distance to nearest patch	0.451	–		
	Position in the cline (319°)	0.130	Position in the cline (319°)	0.2133	0.007
	Patch size	0.163	–		
	Distance to nearest patch	0.453	–		

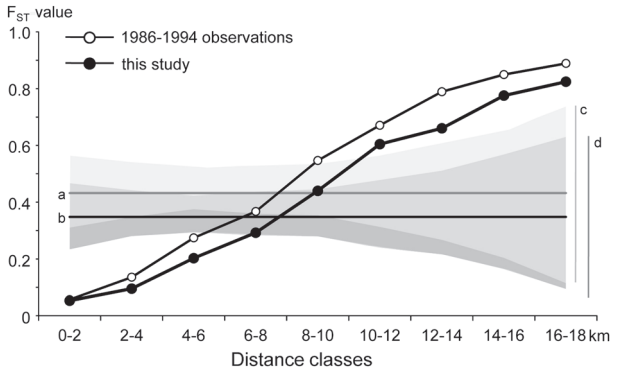

Fig. 3. Spatial autocorrelation analysis describing the geographical pattern of genetic variability at the haemolymph esterase locus of the *Carabus auronitens* populations studied. Genetic differentiation of populations in a specific distance class is measured as pairwise F_{ST} values (Wright 1978), shown as filled circles for the new data set, open circles for the old data set. Mean pairwise F_{ST} values are indicated as lines (a: old data set, b: new data set). Shaded areas show the 95% envelope of F_{ST} distribution under the null hypothesis of spatially random differentiation, obtained after 1000 permutations of the genetic data (c: old data set, d: new data set).

timeframe of only 15 to 20 years we found a change in the population genetic structure of our study species.

The observed changes in the allele frequencies of the locus under study are most likely the result of ongoing gene flow, as other possible factors can be excluded for the following reasons: (1) adaptive processes as a consequence of selective advantages have so far not been found in the populations under study (Drees et al. 2008) and the results of this investigation do not indicate that these are likely to have occurred within the study area. Although such processes can never be ruled out completely, they are unlikely to develop in only 15 to 20 years. (2) Genetic drift is unlikely to cause the observed decrease in population differentiation, as its effects would be the increase of differentiation levels, and would be explained by habitat size (and therefore by population size). The population sizes of *C. auronitens* do not seem to fluctuate strongly in this region as revealed by a long-term study of the population dynamics (Weber and Heimbach 2001). Moreover, the distance between the patches does not influence the amount of genetic change. Instead, the allele frequency changes are dependent on the position of the respective population in the cline and they corroborate the hypothesis of gene flow in flightless *Carabus auronitens*.

The effective gene flow in *C. auronitens* raises the question as to the age and the endurance of the cline. Simulation studies have shown that neutral markers level down in less than 500 generations so that clines can only be found if the secondary contact is recent (Durrett et al. 2000). This is in accordance with the hypothesis of only recent establishment of the investigated cline (Terlutter 1990; Niehues et al. 1996; Drees et al. 2008).

The demonstrable gene flow took place despite the fragmentation of woodlands due to arable fields as well as roads, railway tracks and highways which existed at least during the last 20 years, and usually much longer. Whether the highways or roads are a severe obstacle to dispersal and therefore to gene flow for *Carabus auronitens* (as it was observed for *Carabus violaceus*, another flightless woodland carabid species, Keller and Largiader 2003) or to which amount roads hamper movements of the beetle (cf. Holderegger and Di Giulio 2010) is not subject of this study.

The importance of hedgerows

The gene flow detected in our study is most likely the result of the large number of connecting landscape structures present in the investigation area, such as hedgerows or woodland strips (Davies and Pullin 2007). Connecting landscape elements facilitate dispersal (reviewed by Chetkiewicz et al. 2006) and, thus, allow gene flow (Angelone and Holderegger 2009). Hedgerows act as guidelines for the dispersal of *C. auronitens*, as has been shown in capture–mark–recapture studies and telemetric experiments (Niehues et al. 1996; Drees and Weber 2001). This phenomenon has also been found for other forest carabids (Charrier et al. 1997; Petit and Burel 1998). Recent research also showed that *C. auronitens* is able to use semi-open corridors which form a small-scaled mosaic of heather-dominated vegetation patches and shrubs and groups of trees. In these semi-open habitats *C. auronitens* is able to cross small open habitats to attain woodland (Eggers et al. 2010). This behaviour is likely to occur during rainy nights when the species is more active (Nève 1994).

The amount of gene flow

The repeated investigation of the clinal variation at the esterase-locus in the Westphalian *C. auronitens* populations makes it possible to estimate the effective migration rate (i.e. gene flow) between individual woodland patches in this region, i.e. 0.6% per generation over the last 15 to 20 years. Dispersal rates, estimated on the basis of capture–mark–recapture (CMR) findings in the same area, range from 1.9 to 15.2% with a mean of 7.8% (mean of three years and three woodland–hedge-systems investigated, Drees 2003). The effective migration rate is therefore approximately one tenth of the rate of the observed migration rate, but still has high genetic consequences in the area. Other studies comparing migration rates determined in CMR studies with estimates of the effective migration rate (from genetic investigations) have only been found for one fish (Wilson et al. 2004) and one damselfly species (Watts et al. 2007). The results are

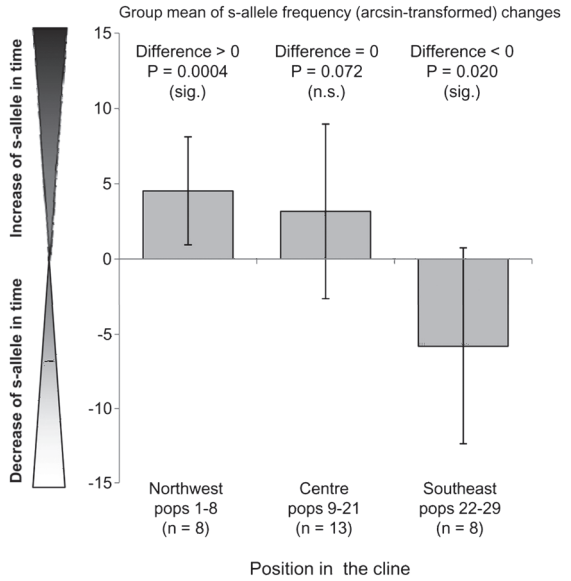

Fig. 4. Temporal changes in the s-allele frequency in different parts of the cline. Populations were grouped according their position in the cline (northwest – centre – southeast, compare Fig. 1 and 2). Group means of s-allele-frequency (arcsin-transformed) difference (old data set minus new data set) and their standard deviation are given. Mean differences were tested against the hypothesis given in the graph using a paired t-test corrected for multiple testing: 'northwest': df = 7, t = 3.5744, significant (sig.); 'centre': df = 12, t = 1.973, not significant (n.s.); 'southeast': df = 7, t = –2.505, significant (sig.).

contradictory, depending on the probability of detecting dispersal and on the relationship between observed long-distance movements and genetically efficient gene flow (cf. Clobert et al. 2001). While Watts et al. (2007) calculate higher effective migration rates on the basis of genetic data, Wilson et al. (2004) found higher migration rates estimated from demographic data.

Consequences for nature conservation

The case of *C. auronitens* shows that, in a connective landscape, corridor features may be effective in enabling exchanges between populations of a flightless, stenotopic woodland species, thus leading to gene flow. This scenario is one conservationists frequently seek to achieve for long-term conservation (Frankel and Soulé 1981), especially in view of ongoing rapid climatic change (De Fonseca et al. 2005). However, implementation is not always easy. The present study shows that a forest carabid beetle may survive in the long term despite a given degree of habitat fragmentation. Connecting landscape structures can have a positive influence, despite the existence of motorways and other fragmenting features – an encouraging example for nature conservation in a fragmented forest context (Meagher 2010). This process is probably variable among forest insects, as their dispersal powers vary greatly (Nève and Baguette 1990). How insect communities are affected by forest fragmentation and hedgerow corridors is still open for research.

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APPENDIX 1

Table A1. *Populations investigated in this study. Numbering according to* Niehues *et al. (1996) is given in brackets. Names, geographic location (UTM coordinates, 32U) and patch sizes (in ha) are given. Data from the old data set taken from* Terlutter *(1990) and* Niehues *et al. (1996). Sample sizes (male/female in brackets) and allele frequencies (s-allele) are given for both data sets. Temporal F_{ST} values ($F_{ST\ obs}$ (*Wright *1978) between the two data sets are shown in the last column.*

Site information					Old data set			New data set		
		Geogr. coordinates								
No.	Name	Xgeo	Ygeo	Size (ha)	Sample year	Sample size (m/f)	Freq s-allele	Sample size (m/f)	Freq s-allele	$F_{ST\ obs}$
1 (101)	Baumberge Natrup	391526	5756618	329	1991/92	36 (19/17)	1.000	33 (18/15)	1.000	0.0000
2 (133)	Amershorst	396031	5757571	143	1992/93	27 (9/18)	0.956	30 (16/14)	0.932	0.0027
3 (83)	Müseler	397279	5757316	17	1991/92	37 (22/15)	0.769	29 (17/12)	0.683	0.0093
4 (4)	Brookbüsche	395241	5754845	43	1986/87	43 (19/24)	0.985	33 (18/15)	0,938	0.0149
5 (93)	Niemann	397803	5754206	23	1991/92	38 (22/16)	0.944	28 (14/14)	0.881	0.0124
6 (37)	Albachten Oberort	398728	5754425	9	1991/92	35 (23/12)	0.851	33 (20/13)	0.761	0.0130
7 (6)	Alvingheide	396622	5753743	35	1986/87	110 (48/62)	0.808	34 (22/12)	0.826	0.0005
8 (7)	Haus Wiek	397537	5752840	22	1986/87	32 (19/13)	0.756	29 (10/19)	0.667	0.0096
9 (112)	Mönninghoff	399705	5751732	8	1991/92	38 (19/19)	0.491	32 (17/15)	0.681	0.0372
10 (118)	Eggemann	400125	5751834	11	1992/93	41 (19/22)	0.698	31 (19/12)	0.721	0.0006
11 (9)	Lövelingloh	401422	5752203	23	1985/86	143 (66/77)	0.827	31 (16/15)	0.696	0.0236
12 (98)	Holtschulte	402762	5752189	79	1991/92	35 (14/21)	0.643	30 (17/13)	0.605	0.0015
13 (8)	Forst Tinnen	407750	5751245	86	1986/87 1989/90	211 (126/85)	0.740	49 (28/21)	0.586	0.0265
14 (8b)	Forst Tinnen west	399797	5750953	86	1992/93	44 (23/21)	0.585	33 (15/18)	0.529	0.0032
15 (34)	Gut Herding	404746	5750871	26	1990/91	33 (9/24)	0.316	35 (17/18)	0.189	0.0186
16 (42)	Wegert	398759	5749992	72	1991/92	40 (30/10)	0.500	29 (18/11)	0.400	0.0101
17 (44)	Schachtrup	399612	5750365	72	1991/92	37 (19/18)	0.491	30 (16/14)	0.409	0.0068
18 (54)	Mielingloh	404391	5750181	20	1991/92	46 (17/29)	0.453	34 (17/17)	0.392	0.0038
19 (10)	Ventruper Heide	398680	5749566	92	1986/87	26 (12/14)	0.400	30 (7/23)	0.453	0.0029
20 (43)	Ventruper Heide SW	397527	5748265	31	1991/92	35 (16/19)	0.574	28 (13/15)	0.535	0.0015
21 (38)	Potthoffsheide	397168	5747384	37	1991/92	36 (13/23)	0.644	33 (21/12)	0.489	0.0245
22 (126)	Davert Druffel	406184	5746836	746	1992/93	34 (14/20)	0.056	35 (14/21)	0.036	0.0023
23 (16)	Davert Venne Ost	401508	5746267	411	1986/87 1990/91	55 (35/20)	0.107	35 (18/17)	0.096	0.0023
24 (50)	Davert Bönnewegbach	402342	5746262	106	1991/92	36 (17/19)	0.109	38 (18/20)	0.121	0.0004
25 (138)	Davert Klosterholz West	401572	5744933	411	1992/93	30 (13/17)	0.021	35 (17/18)	0.132	0.0436
26 (143)	Davert Klosterholz	402361	5745563	106	1993/94	36 (15/21)	0.070	33 (22/11)	0.250	0.0603
27 (13)	Davert Ost	407134	5745206	746	1986/87	65 (43/22)	0.046	34 (17/17)	0.118	0.0172
28 (15)	Davert Hof Rolf	405614	5743383	746	1986/87 1991/92	82 (49/33)	0.000	59 (25/34)	0.032	0.0163
29 (18)	Davensberg	401990	5742232	54	1986/87 1990/91	39 (22/17)	0.107	34 (14/20)	0.148	0.0038