



Are peripheral populations special? Congruent patterns in two butterfly species

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Populations at range margins may be genetically different from more central ones for a number of mutually non-exclusive reasons. Specific selection pressures may operate in environments that are more marginal for the species. Genetic drift may also have a strong effect in these populations if they are small, isolated and/or have experienced significant bottlenecks during the colonisation phase. The question if peripheral populations are special, and if yes then how and why, is of obvious relevance for speciation theory, as well as for conservation biology. To evaluate the uniqueness of populations at range margins and the influence of gene flow and selection, we performed a morphometric study of two grassland butterfly species: *Coenonympha arcania* and *C. hero* (Lepidoptera: Nymphalidae). The samples were collected from Swedish populations that are peripheral and isolated from the main area of the species distributions and from populations in the Baltic states that are peripheral but connected to the main area of the species distributions. These samples were compared to those from central parts of the species distributions. The isolated populations in both species differed consistently from both peripheral and central populations in their wing size and shape. We interpret this as a result of selection caused by differences in population structure in these isolated locations, presumably favoring different dispersal propensity of these butterflies. Alternative explanations based on colonisation history, latitudinal effects, inbreeding or phenotypic plasticity appear less plausible. As a contrast, the much weaker and seemingly random among-region differences in wing patterns are more likely to be ascribed to weaker selection pressures allowing genetic drift to be influential. In conclusion, both morphological data and results from neutral genetic markers in earlier studies of the same system provide congruent evidence of both adaptation and genetic drift in the isolated Swedish populations of both species.

Peripheral populations at the margin of a species' range can act as evolutionarily hotspots (Mayr 1954, Levin 1970, Lesica and Allendorf 1995). A high degree of differentiation due to their more marked exposure to diversifying processes – random genetic drift and selection – can eventually lead to speciation and thus increases the value of these populations in the context of biodiversity (Lesica and Allendorf 1995, Channell and Lomolino 2000). However, despite the fact that the peripheral populations will increase the evolutionary potential of the species as a whole, having a peripheral position may constrain evolvability of the particular populations and increase their extinction risk. This is because the frequently reported reduced levels of genetic variation and biological constraints at range margins adversely affect the potential to adapt to the environment (Jones et al. 1968, Kirkpatrick and Barton 1997, Gilchrist et al. 2000, Bridle and Vines 2007). Nevertheless, lower levels of genetic variation in peripheral populations is not the only possible outcome (i.e. Van Rossum et al. 2003,

Gapare et al. 2005), and the type and strength of selection in action also determine the fate of these units (Wilson et al. 1991, Hoffmann and Blows 1994, van Buskirk and Willi 2006, Bridle and Vines 2007).

In particular, selection pressures may be more pronounced at range edges compared to more central parts due to more extreme conditions and/or larger fluctuations in environmental conditions. Unique conditions in combination with isolation may select for particular genotypes different from more central ones (i.e. Santamaria et al. 2003) and may also reduce overall variability (Bulmer 1971, Roff 1997). On the other hand, larger fluctuations in marginal habitats can increase variability in character values if there is temporal variation in selection pressures over time (Helgadóttir and Snaydon 1986).

In addition to selection caused by atypical environmental conditions, a peripheral position may create predictable specific selective pressures related to different (meta) population and landscape structures (Travis and Dytham

1998, Heino and Hanski 2001). Selection may favor a higher dispersal ability in a more fragmented landscape if the cost for allocating resources in flight capacity is outweighed by the benefits of finding a suitable patch (Taylor and Merriam 1995, Thomas et al. 1998, Hill et al. 1999). However, if the mortality of the disperser is increased as fragmentation increases (den Boer 1990, Schtickzelle et al. 2006) selection will act in the opposite direction (i.e. Dempster 1991). Also variation in matrix quality can alter the individuals behavior (Haynes and Cronin 2006). For example, Ricketts (2001) found that four out of six studied butterfly species were less reluctant to leave a patch surrounded by more hostile matrix. Thus, if changes in landscape configuration affect the selection pressure on flight behavior then, in the case of flying insects, changes in wing configuration can be expected (Betts and Wootton 1988, Wickman 1992, Berwaerts et al. 2002, Fric and Konvička 2002, Dhuyvetter et al. 2007), especially because the heritability of dispersal related traits has repeatedly shown to be significant (reviewed in Roff and Fairbairn 2001).

The relative influence of drift and selection in peripheral populations can be separated when isolated peripheral populations are compared to peripheral ones still in contact with the main area of distribution. The Scandinavian Peninsula being compared to the Baltic states provide a good opportunity for such studies. Scandinavia is separated from other terrestrial areas by the Baltic Sea in the east and south, and by the Atlantic Ocean in the west, which causes an isolated status for many populations found there. Some of such species have a wide global distribution, with the Scandinavian populations being both peripheral and isolated, while the populations on the eastern coast of the Baltic Sea, just a few hundred kilometers apart, are peripheral but usually connected to the species main area of distribution.

The populations of two closely related butterflies, the scarce heath *Coenonympha hero*, and the pearly heath *C. arcania*, in Scandinavia and the Baltic states are both peripheral relative to the extensive total ranges of these species. The populations in Scandinavia, but not those in the Baltic states, are also isolated from the main bodies of both species ranges (Fig. 1a, b). In previous studies it was found that neutral genetic variability on allozyme level was significantly reduced in the Swedish populations compared to both peripheral and central populations (Cassel and Tammaru 2003, Besold et al. 2008b). There was also a higher level of differentiation between the Swedish and Baltic populations than between the Baltic and central populations despite the fact that the Swedish and Baltic populations are an order of magnitude closer in distance than the Baltic and the central populations (Cassel and Tammaru 2003, Besold et al. 2008b).

The previous studies were based on presumably neutral genetic markers (Besold et al. 2008a) and the data allowed estimates of the effects of genetic drift. However, the nature of the data did not allow conclusions on the influence of selection as a diversifying agent. To fill this gap, we have here analysed corresponding geographical patterns in morphological traits in which at least some differences can be expected to have adaptive causes. By studying wing morphology and wing pattern in the two *Coenonympha*

species, we addressed the following questions: 1) is the genetic uniqueness of the isolated Swedish populations found for allozymes also reflected in morphological traits? 2) Are the reduced levels of genetic variation also reflected in reduced levels of variances of morphological traits? 3) If yes, which evolutionary processes have caused the pattern?

To answer these questions we measured ten different wing traits and compared the values among populations from three biogeographically different regions; one central (central Russia for *C. hero* and central Europe for *C. arcania*), one peripheral but connected to the main area of distribution (the Baltic states for both species) and one peripheral and isolated from the rest of the main distribution (Sweden for both species). The two *Coenonympha* species, closely related but with clear differences in their biology, can be seen as two replicates within one study frame. Any congruent pattern detected will add valuable strength to the conclusions by compensating for a common weakness in these kind of studies – the absence of replication (Eckert et al. 2008). An additional strength of this study design is that we can evaluate the patterns found not only as dependent on the relative locations of the populations but also on their history which could be inferred from respective allozyme data (Cassel and Tammaru 2003, Besold et al. 2008b), an aspect which is also largely lacking in studies on central versus peripheral populations (Eckert et al. 2008).

Material and methods

Biological background

The scarce heath butterfly *Coenonympha hero* (Nymphalidae: Satyrinae) and the pearly heath butterfly *C. arcania* are both relatively small butterflies with wingspans of 26–32 mm and 28–34 mm, respectively (Eliasson 2005b). Both are slow-flying species on the wings from mid June to mid July in northern Europe. For *C. arcania* the flight period extends from late May until mid September in southern Europe. Both species are generally univoltine, although in recent years partial second broods of *C. arcania* have been observed (Kadlec and Vrba 2007). Both species are found in grasslands and open warm woodlands (Higgins and Riley 1993) as well as in clear cuttings and in glades along roads

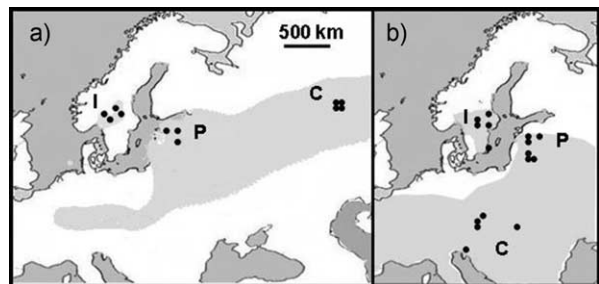


Figure 1. Distribution range (marked in grey) of (a) the scarce heath butterfly *Coenonympha hero* and (b) the pearly heath butterfly *Coenonympha arcania* in Europe and western Asia. Black dots indicate sampling sites of both species representing central (C), peripheral (P) and isolated (I) parts of their distributions.

(Eliasson 2005b), although *C. hero* prefers more hygrophilous conditions than *C. arcania*. Due to the differences in habitat requirements the species only rarely co-occur. Females of both species lay their eggs singly on dry vegetation close to the ground and scattered on the habitat patch. The larvae of both species feed on various species of grass (Tolman and Lewington 1998, Eliasson et al. 2005) and they generally hibernate in the 3rd instar resting on dry vegetation close to the ground. Growth resumes in spring and the 5th instar larvae pupate around the end of May or early June. The pupae are found on dry vegetation close to the ground.

Species distributions

Coenonympha hero is a widespread, although locally threatened species (Berglund 2005) with a distribution ranging from Japan in the east, through central Russia to central Europe in the west. The distribution is fragmentary in western and northern Europe and the species is considered threatened in most countries (van Swaay and Warren 1999). The populations in Scandinavia are isolated from the main body of the species' range (Fig. 1a), and the species is classified as "near threatened" on the Swedish Red list (Gärdenfors 2005). In the Baltic states its distribution varies from rare and local to widespread (Sulcs and Viidalepp 1974, Viidalepp 1995, Ivinskis 2004).

Coenonympha arcania has a similar distribution as *C. hero* with peripheral populations in the Baltic states and Scandinavia (Fig. 1b), with the difference that it is widely distributed in Europe, from the northern Mediterranean to south-central Scandinavia and central Estonia. In the east, it is found in Turkey, Transcaucasia and in southern Russia and central parts of the Ural Mts and Transcaucasia (Tolman and Lewington 1998, Kudrna 2002, Eliasson 2005a).

Sampling

Adults were collected in the field during the beginning or at the peak of the flight period in one or two field seasons per location. We selected sample sites so that peripheral and isolated (Sweden: Eliasson 2005a), peripheral and connected (Baltic countries: Kesküla 1992, Ivinskis 2004) and central parts (central Europe for *C. arcania*: Kudrna 2002, central Russia for *C. hero*: Cassel and Tammaru 2003) were represented. Samples of 4–41 field collected males per population were measured, the median sample size being 26 for *C. arcania* and 27 for *C. hero* (Table 1). We included only males in the analyses as the number of collected females was too low for a meaningful analysis, and both species show sex dimorphisms in the traits measured.

Wing traits and measurements

Wings were detached from the body before measurement, by carefully cutting their connection to the body at the base of the wings. A total of ten traits were measured (Fig. 2). These were 1) area of fore and 2) hind wing, 3) length of fore and 4) hind wing, measured from the wing base to the tip of the

wing, 5) width of fore and 6) hind wing, size of the black 7) and the white 8) part of the third eyespot on the hind wing, 9) area of the white band on the hind wing and 10) distance between the first eye spot on the hind wing and the wing base. Measurements were made with an image analyser made of a video camera coupled to a computer. This allows for very precise measurements of butterfly wings (Windig 1991). The image analyser consisted of a JVC TK 5066E Camera and a Leica image analysis system. Measurements were performed semi-automatically with the help of a program written by JJW in the Qwin image analysis software (produced by Leica). In order to calibrate the equipment in the beginning of each measurement session settings were adjusted against one particular wing. The program automatically thresholded a wing from the background for the area measurements (= number of pixels converted to mm²). Start and endpoints for width and length were indicated manually on the monitor. Both were more than 300 pixels in length so that pixellation error (warned for in Palmer 1994) was not large. Area of eyespots and white band were measured by first thresholding black (white) colour from the rest of the image, and then manually indicating which black (white) part or parts made up the black ring (white core) of the eyespot. Measurement results were sent to a spreadsheet without the person operating the program being able to inspect them.

Statistical analyses

We analysed traits related to wing morphology as dependent on region (a three-level variable: "isolated", "peripheral" and "central") using general linear models with random effects (SAS PROC MIXED, Littell et al. 1996). In all analyses, population (sample site) was considered a random variable, and Kenward-Roger method (Littell et al. 2002) was applied to determine the number of denominator degrees of freedom. This ensured that a local population (and not each individual) was considered an independent observation which allowed us to avoid overestimating degrees of freedom. Tukey tests were used for pairwise comparisons between the three regions.

No further variables were included in the model when wing size was the dependent variable. For the analyses of wing shape we set wing length as a dependent variable and wing width as a covariate, i.e. we asked if wing length adjusted for wing width was different between the regions. Similarly, the differences in the relative area of the two wings were tested with forewing area as the dependent variable, and hind wing area as a covariate. When looking for among-region differences in the size of wing pattern elements, we included the area of the respective wing as a covariate to correct for elementary allometric effects.

To examine "pure" latitudinal trends, i.e. those presumably unaffected by isolation, we performed a combined analysis of the central and peripheral regions of *C. arcania*. All the variables previously analysed as dependent on region were now analysed as dependent on the latitude of the collection site. Once again, we treated population and not individual as the unit of observation. Accordingly, "population" was considered a random variable, and Kenward-Roger method was used to adjust denominator degrees of freedom

Table 1. Sampled locations, sampling year and sample sizes (n) included in the study on morphometric differences between central, peripheral and isolated populations of the pearly heath butterfly *C. arcania* and the scarce heath butterfly *C. hero*.

Sampling location	n	Sampling year	Country of origin	Longitude	Latitude
<i>C. arcania</i>					
Central					
Kurdejev	7	2004	Czech Republic	16°45'E	48°58'N
Malinky	8	2004	Czech Republic	17°10'E	49°10'N
Mohelno	4	2004	Czech Republic	16°11'E	49°06'N
Tesanky	9	2004	Czech Republic	17°17'E	49°16'N
Monastirishte	41	2004	Bulgaria	23°09'E	43°03'N
Zdanice	18	2004	Czech Republic	17°01'E	49°04'N
Podnanos	42	2004	Slovenia	13°59'E	45°48'N
Peripheral					
Koikküla	27	2004	Estonia	26°17'E	57°36'N
Kurepalu	30	2004	Estonia	26°50'E	58°19'N
Veski	27	2004	Estonia	27°32'E	57°50'N
Kavarskas	30	2004	Lithuania	24°57'E	55°27'N
Raseiniai	25	2004	Lithuania	23°26'E	55°19'N
Trakai	30	2004	Lithuania	24°31'E	54°35'N
Isolated					
Felingen	27	2004	Sweden	16°00'E	59°30'N
Grinduga	25	2004	Sweden	17°10'E	60°23'N
Morgendal	27	2004	Sweden	16°03'E	59°21'N
Torslunda	15	2004	Sweden	16°20'E	56°22'N
Bråförs	36	2004	Sweden	15°44'E	60°02'N
Ultuna	21	2004	Sweden	17°24'E	59°29'N
<i>C. hero</i>					
Central					
Sportivnaya	18	1998	Russia	60°05'E	56°50'N
Il'movka	23	1998	Russia	59°35'E	56°48'N
Krasnoyar	33	1998	Russia	59°50'E	56°37'N
Merkitasikha	32	1998	Russia	59°22'E	57°08'N
Peripheral					
Sonda	7	1998	Estonia	26°49'E	59°23'N
Tähtvere	28	1999	Estonia	26°39'E	58°24'N
Laelatu	18	1998	Estonia	23°33'E	58°35'N
Isolated					
Brunsborg	27	1996, 1998	Sweden	12°56'E	59°37'N
Fallet	27	1996, 1998	Sweden	13°29'E	59°27'N
Hagge	27	1997, 1998	Sweden	15°20'E	60°07'N
Östmark	14	1996	Sweden	12°45'E	60°16'N

correspondingly. Moreover, we added latitude a covariate to the core ANOVA models to check for possible changes in the results. For *C. hero*, among-region differences in the latitudes of sampling sites were considered too limited to allow for a meaningful analysis.

To find out if individual variability in trait values was different between the three regions, we calculated CV values of each analysed trait for each population. One-way ANOVAs with these population-specific values were performed to test for among-region differences in the relative variability of the traits. In search for general patterns, we combined such trait-specific data sets, and the population-specific CV's were analysed by a two-way ANOVA with "region" and "trait" (treated as random) as independent factors.

Results

Wing morphology traits

The two species studied displayed largely consistent among-region patterns in wing morphology (Fig. 3a–j). In both

species, both hind and fore wings were smaller in the isolated populations (Sweden) compared to the central populations, with no significant differences between the peripheral and central populations (except for the hind wing in *C. arcania* where all three regions were significantly differentiated, Fig. 3b). Similarly, in the isolated area, both wings in both species were shorter relative to their widths, with no significant differences between the peripheral and central populations (Fig. 3c–d for *C. hero* and h–i for *C. arcania*). Again, the hind wing shape differed significantly between all regions for *C. arcania* (Fig. 3h). For *C. arcania* hind wings were smaller for a given forewing size in the isolated populations compared to the central (Fig. 3j). There was a tendency for such a difference also in *C. hero* although statistical significance was not attained (Fig. 3e).

Wing pattern traits

The elements of wing pattern showed much weaker tendencies, and largely inconsistent differences between the regions (Fig. 4a–h). White eye spot and black eye spot were both smallest in the isolated populations of *C. arcania*

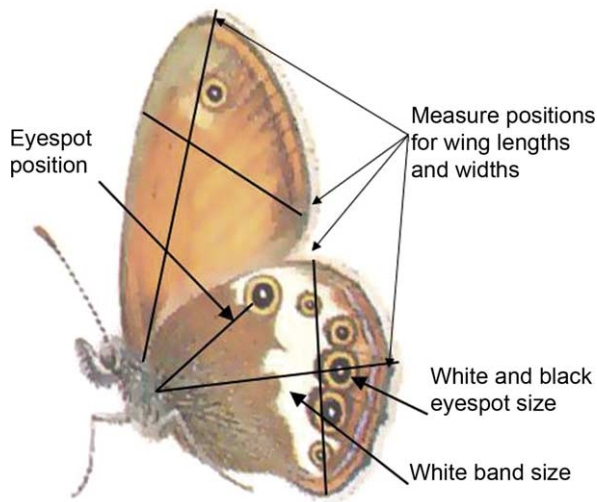


Figure 2. Wing traits measured on males of *Coenonympha hero* and *C. arcania* originating from central, peripheral and isolated parts of their distribution. Traits include area (not indicated), length and width of both fore and hind wings, size of one eye spot (both the black and white part), size of the white band and the position of the first eye spot.

and largest in the central ones (Fig. 4e–f). In *C. hero*, the only significant difference was in the size of the white band which was the widest in the isolated Swedish populations and did not differ between the peripheral and central populations (Fig. 4c). The sizes of the wing pattern elements were not strongly correlated with each other so it was not considered meaningful to apply principal component analysis.

Latitudinal trends

In the data set combining *C. arcania* trait values for central and peripheral regions, a significant ($p < 0.05$) latitudinal trend was detected only for hind wing shape ($F_{1,8.36} = 15.9$, $p = 0.0037$, shorter for given width moving northwards). However, a closer examination revealed that there was no indication of a latitudinal trend within either of the two regions ($p > 0.4$): the pattern was thus confined to the difference between the two regions which makes it ambiguous to ascribe the pattern to a latitudinal trend. In consistence with the limited evidence of latitudinal trends, adding “latitude” as a covariate to the core analyses (Fig. 3, 4) resulted in no qualitative changes.

Variability in trait values

In *C. hero*, among-region differences in population-specific CV did not reach significance ($p > 0.05$) for any of the wing pattern traits (white spot size, black spot size, white band size and spot position). However, there was still some regularity in the pattern: for three traits out of four (not in spot position), the central area was the most variable one. Moreover, for three traits out of four (not in white band size), the peripheral area was more variable than the isolated area. It was therefore considered meaningful to combine the

data sets for different traits to increase statistical power. Indeed, a two-way ANOVA with region and trait as independent variables detected a significant among-region difference in the levels of population-specific variability ($F_{2,38} = 4.34$, $p = 0.020$, values of CV logarithmically transformed to stabilise variances, initially unequal for different traits). The isolated region proved to be least variable of the three, the central region was most variable, with a significant difference between these two (Tukey-Kramer adjustment, $t = 2.82$ DF = 38, $p = 0.020$).

In *C. arcania*, the pattern was similar in the fact that, in three traits (black spot size, white band size and spot position) least variation was found in the isolated region though in no case was significance attained. White spot size formed an exception with the isolated region being most variable (one-way ANOVA: $F_{2,18} = 4.92$, $p = 0.021$). In the combined analysis of the four different traits (see above for details), the isolated region was the least variable, but not significantly so ($F_{2,70} = 0.75$, $p = 0.47$).

An analogous analysis of morphological traits (areas and shapes of both wings, for both species) revealed no consistent patterns, neither were any among-region differences significant ($p > 0.5$, for most traits). A combined analysis of these traits was not considered meaningful as the values of these traits are not independent.

Discussion

We found significant and congruent among-region differences in means of wing size and shape for both species. More specifically, there were pronounced differences between the individuals from the isolated region on the one hand, and those from both the peripheral and central region on the other (Fig. 3a–j). Individuals from the peripheral region differed only marginally from the central ones despite that the distances between the peripheral and central collection sites are an order of magnitude larger than the distances between peripheral and isolated sites. The results are also noteworthy when considering that the peripheral and isolated populations are found on comparable latitudes, while the populations of the central regions are situated further south (particularly for *C. arcania*). These results agree well with respective genetic data showing larger differentiation between the peripheral and isolated populations than between the peripheral and central populations. We will argue that the pattern in the wing morphology traits is primarily due to differences in population structure. Explanations based on climatic effects, phenotypic plasticity, colonisation history or in-breeding appear less plausible, as discussed in more detail below.

The populations of both species appears to be more fragmented in Sweden than in the Baltic states. Sweden has a high proportion of forest due to an intensive afforestation the last century (Nilsson 1990), while Estonia for example has not changed in landscape configuration considerably during the same time period (Palang et al. 1998). Further, due to a significant decline in low intensive agriculture in Sweden the past century (Dahlstrom et al. 2006), the proportion of semi-natural grasslands (primary habitats of *Coenonympha* species (Eliasson et al. 2005)) is definitely

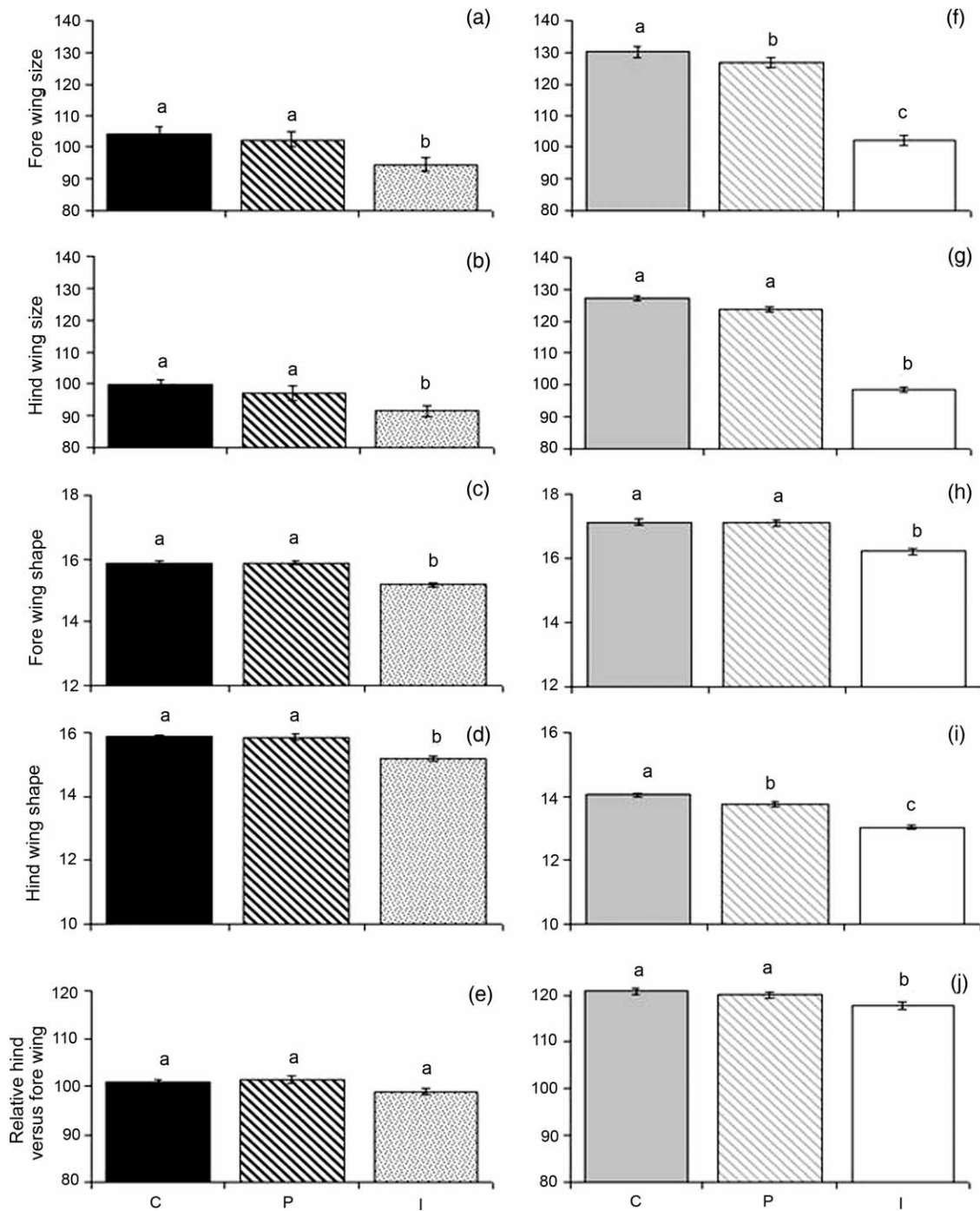


Figure 3. Wing trait means in (a–e) *Coenonympha hero* and (f–j) *C. arcania* from three biogeographical regions (central (C) as dark bars, peripheral (P) as striped bars and isolated (I) as light bars) presented as LS means (\pm SE) from respective ANOVA models (see Methods for details). Mean values marked with an identical letters were not statistically significant ($p > 0.05$).

lower in Sweden compared to the Baltic states. This suggests that 1) the amount of available habitat is lower for the Swedish butterflies with the average distance between habitat patches being larger, and 2) that the matrix separating habitat patches are more hostile. As a consequence, both *Coenonympha* species occur to a larger extent as isolated populations (as documented for *C. hero* in Cassel-Lundhagen and Sjögren-Gulve 2007, Cassel-Lundhagen et al. 2008), whereas the distribution approaches a continuous one in at least parts of Estonia (unpubl.).

Significant isolation in combination with a matrix of low permeability is expected to select against dispersive genotypes if the mortality of migrants is significant (den Boer 1990, Cody and Overton 1996, Heino and Hanski 2001, Schtickzelle et al. 2006). Altered behavior as a response to selection against migrants is likely reflected also in their phenotype (Davis 1984, Dempster 1991, Thomas et al. 1998, Hill et al. 1999, Berwaerts et al. 2002, 2006, Fric and Konvička 2002, Merckx and Van Dyck 2006), in good consistence with the patterns revealed in this study. The

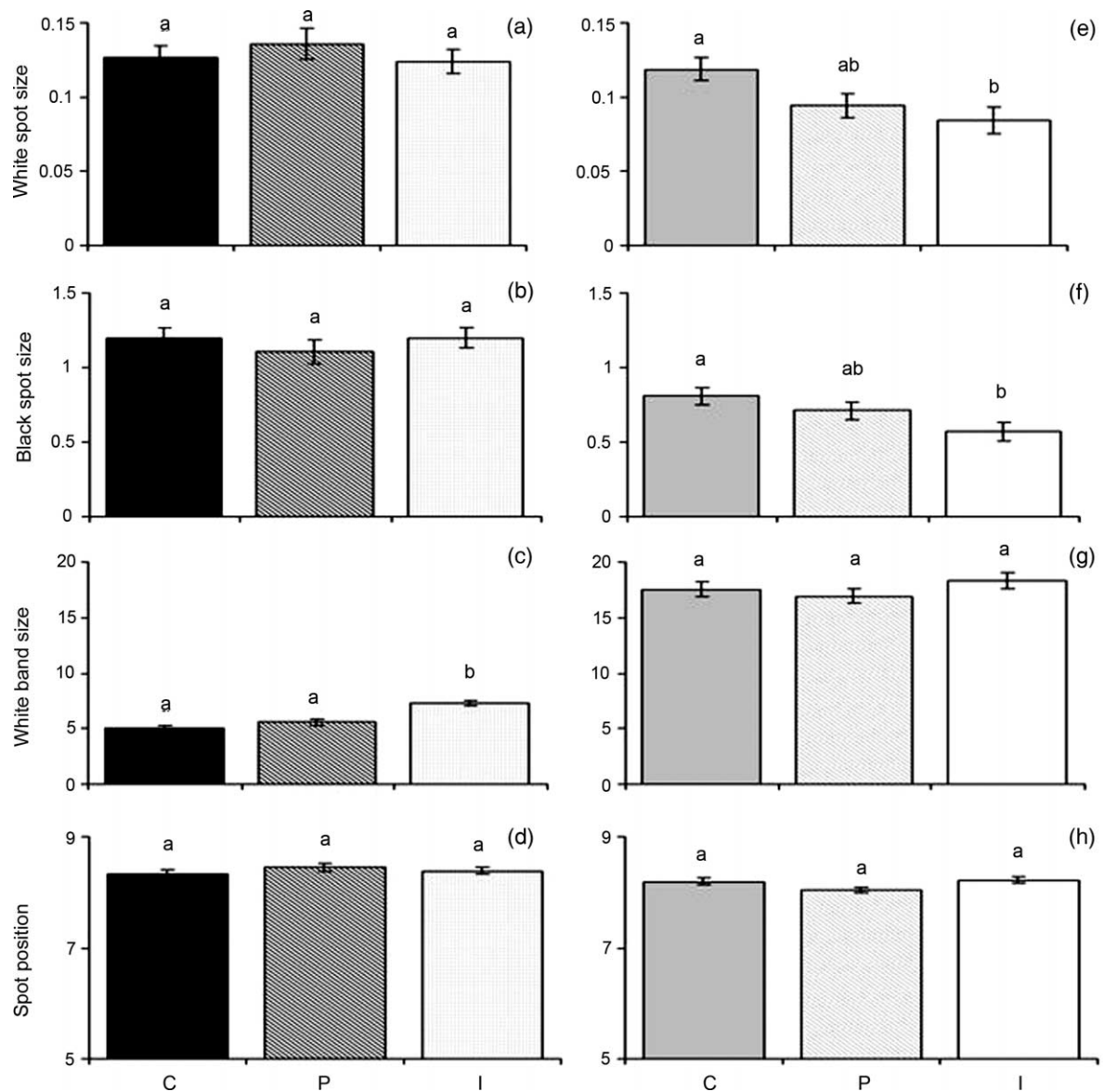


Figure 4. Wing pattern means in (a–d) *Coenonympha hero* and (e–h) *C. arcania* from three biogeographical regions (central (C) as dark bars, peripheral (P) as striped bars and isolated (I) as light bars) presented as LS means (\pm SE) from respective ANOVA models (see Methods for details). Mean values marked with an identical letters were not statistically significant ($p > 0.05$).

correlation between wing morphology and body measures is not straight forward however (Van Dyck and Wiklund 2002), and flight performance depends on a combination of both wing and body traits (Berwaerts et al. 2006). Short and broad wings (like revealed here in the Swedish *Coenonympha* males) are nevertheless expected to favor slow, agile flight and to be more common among forms that fly in restricted spaces and dense vegetation (Betts and Wootton 1988). Further, since both *C. arcania* and *C. hero* are species with patrolling males in Sweden (Wickman 1992), thus using a significant time searching the patch for available females, we can expect that both patch configuration and matrix type adjacent to the patches will influence the males behavior (Stamps et al. 1987, Ricketts 2001, Schultz and Crone 2001, Haynes and Cronin 2006). Our hypothesis is, thus, well explainable for the differences found between

Sweden and Estonia. However, in order to confirm our conclusions we need to test them on areas which differ in population structure also within more central parts of the species distributions.

If the differences in the isolated Swedish populations were to be explained by a selection event during the colonisation phase, we would expect to find the opposite to what we observe (larger and longer wings rather than smaller and more rounded). This is because, irrespective of the exact colonisation route to Sweden, the dispersers had to actively fly or to be carried by the wind over open water, thus conducting more or less long distance dispersal. Better dispersers among butterflies are expected to be larger and to have more pointed wings (Kölliker-Ott et al. 2004, Dockx 2007) rather than smaller with more rounded wings as was found here. It should be noted though, that we have

measured only males and as the founders were most likely only females their phenotype may not necessarily mirror that of the males (Saastamoinen 2007).

In insects, traits related to body size and shape do frequently respond to varying temperature in a systematic way. In particular, controlled experiments have shown effects of temperature on both wing size (Azevedo et al. 1998, Bitner-Mathé and Klaczko 1999, Bernardo et al. 2007) and wing shape (Azevedo et al. 1998). As most environmental effects of significance for insects co-vary with latitude (Nylin and Svärd 1991, Chown and Gaston 1999, Fischer and Fiedler 2002), geographic trends in morphological traits can, in principle, be based on phenotypic plasticity (Azevedo et al. 1998). However, we did not find any correlation between studied morphological traits and latitude. This argues against pure environmental plastic or adaptive effects due to climate. Given the proximity and climatic similarity between Scandinavia and the Baltic states, an environment-based explanation between these regions appears less likely. The lack of differences among the peripheral and central, despite the marked differences in latitude, gives this argument further strength. Thus, the absence of a latitudinal trend supports our conclusions that the observed differences are of genetic nature, and not an expression of plasticity. Finally, we have not found any study reporting an effect of temperature on relative size between fore and hind wings, a character we found differing among the regions (Fig. 3e, j).

Inbreeding depression can also lead to both decreases in body size (Bryant et al. 1986, but see Saccheri et al. 2001) and changed wing shapes (Kölliker-Ott et al. 2003). However, there are no indications of inbreeding depression in these species within Sweden, neither reflected as elevated levels of fluctuating asymmetry (FA) (Windig et al. 2000) nor as fixed deleterious alleles (Cassel et al. 2001). Cassel et al. (2001) found that females of *C. hero* originating from large Swedish populations had both high egg hatching rates and high offspring survival, whereas both hatching rates and survival was significantly reduced in small and isolated populations of the same species.

The consistently lower variances (although not significant in all cases) for the wing pattern traits mirrors the reduced allozyme variation for both species in the isolated populations (Cassel and Tammaru 2003, Besold et al. 2008b). Although the morphometric measurements were done on field collected specimens and during different number of flight seasons (that may inflate the environmental effect (Talloon et al. 2008)) the pattern was congruent among species and traits, thus supporting a significant genetic effect. If environmental effects had contributed significantly to the differences in variation, we would expect the isolated populations of *C. hero* to have larger rather than lower variances than the populations in the other two regions due to that the populations from the peripheral and central region were sampled within one season, while the isolated were sampled in three (Table 1).

The consistent among-region patterns found for wing size and shape were not mirrored in the wing pattern traits (Fig. 4a–h). We found no clear concordance within traits between species nor within species between traits. From the lack of significant differences among regions we conclude

that these characters experience similar selection pressures in all regions. The occasional significant differences found (the white band for *C. arcania* and the eye spot for *C. hero*) and the lack of congruence between species confirm that the traits are also, to some extent, influenced by genetic drift and evolve independently from each other (Brakefield 2001). The, although limited, evidence for genetic drift in wing pattern traits may also be seen as additional support for the primarily adaptive nature of the differences in the morphology traits.

In conclusion, it appears that the isolated Swedish populations experience not only a lack of gene flow from more central parts of these two species distributions (Cassel and Tammaru 2003, Besold et al. 2008b), but also conditions that are unique that create congruent and diversifying selection pressures on both species. If the differences observed reflect differences in flight performance, we could also expect these peripheral populations to differ in reactivity to climate change (Warren et al. 2001). Thus, this system of peripheral populations that differ in genetic variability and morphology creates a unique opportunity to study the influences of these factors on responses to increased temperature and changed landscape structures. The variation documented may also ultimately lead to speciation, even if the time passed since the last glaciation may still be too short for new butterfly species to form in northern Europe.

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References

- Azevedo, R. B. R. et al. 1998. Latitudinal variation of wing: thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. – *Evolution* 52: 1353–1362.
- Berglind, S.-Å. 2005. *Coenonympha hero* Linné 1761, scarce heath. Species fact sheet. – In: Gärdenfors, U. (ed.), Rödlistade arter i Sverige – The 200 Swedish Red List of Swedish Species. Swedish Species Information Centre, SLU, in Swedish, <<http://www.artdata.slu.se>>.
- Bernardo, U. et al. 2007. Phenotypic plasticity of pigmentation and morphometric traits in *Pnigalio soemius* (Hymenoptera: Eulophidae). – *Bull. Entomol. Res.* 97: 101–109.
- Berwaerts, K. et al. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. – *Funct. Ecol.* 16: 484–491.
- Berwaerts, K. et al. 2006. On the sex-specific mechanisms of butterfly flight: flight performance relative to flight morphology, wing kinematics, and sex in *Pararge aegeria*. – *Biol. J. Linn. Soc.* 89: 675–687.

- Besold, J. et al. 2008a. Allozyme polymorphisms in the small heath, *Coenonympha pamphilus*: recent ecological selection or old biogeographical signal? – *Ann. Zool. Fenn.* 45: 217–228.
- Besold, J. et al. 2008b. Strong genetic impoverishment from the centre of distribution in southern Europe to peripheral Baltic and isolated Scandinavian populations. – *J. Biogeogr.* 35: 2090–2101.
- Betts, C. R. and Wootton, R. J. 1988. Wing shape and flight behaviour in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): a preliminary analysis. – *J. Exp. Biol.* 138: 271–288.
- Bitner-Mathé, B. C. and Klaczko, L. B. 1999. Plasticity of *Drosophila melanogaster* wing morphology: effects of sex, temperature and density. – *Genetica* 105: 203–210.
- Brakefield, P. M. 2001. The structure of a character and the evolution of patterns. – In: Wagner, G. P. (ed.), *The character concept in evolutionary biology*. Academic Press, pp. 343–361.
- Bridle, J. R. and Vines, T. H. 2007. Limits to evolution at range margins: when and why does adaptation fail? – *Trends Ecol. Evol.* 22: 140–147.
- Bryant, E. H. et al. 1986. The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. – *Genetics* 114: 1191–1211.
- Bulmer, M. G. 1971. The effect of selection on genetic variability. – *Am. Nat.* 105: 201.
- Cassel, A. and Tammaru, T. 2003. Allozyme variability in central, peripheral and isolated populations of the scarce heath (*Coenonympha hero*: Lepidoptera, Nymphalidae); implications for conservation. – *Conserv. Genet.* 4: 83–93.
- Cassel, A. et al. 2001. Effects of population size and food stress on fitness-related characters in the scarce heath, a rare butterfly in western Europe. – *Conserv. Biol.* 15: 1667–1673.
- Cassel-Lundhagen, A. and Sjögren-Gulve, P. 2007. Limited dispersal by the rare scarce heath butterfly – potential consequences for population persistence. – *J. Insect Conserv.* 11: 113–121.
- Cassel-Lundhagen, A. et al. 2008. Effects of patch characteristics and isolation on relative abundance of the scarce heath butterfly *Coenonympha hero* (Nymphalidae). – *J. Insect Conserv.* 12: 477–482.
- Channell, R. and Lomolino, M. V. 2000. Dynamic biogeography and conservation of endangered species. – *Nature* 403: 84–86.
- Chown, S. L. and Gaston, K. J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. – *Biol. Rev.* 74: 87–120.
- Cody, M. L. and Overton, J. M. 1996. Short-term evolution of reduced dispersal in island plant populations. – *J. Ecol.* 84: 53–61.
- Dahlstrom, A. et al. 2006. The history (1620–2003) of land use, people and livestock, and the relationship to present plant species diversity in a rural landscape in Sweden. – *Environ. Hist.* 12: 191–212.
- Davis, M. 1984. The flight and migration ecology of the red milkweed beetle (*Tetraopes tephthalmus*). – *Ecology* 64: 230–234.
- Dempster, J. P. 1991. Fragmentation, isolation and mobility of insect populations. – In: Collins, N. and Thomas, J. (eds), *The conservation of insects and their habitats*. Academic Press, pp. 143–153.
- den Boer, P. J. 1990. The survival value of dispersal in terrestrial arthropods. – *Biol. Conserv.* 54: 175–192.
- Dhuyvetter, H. et al. 2007. Differentiation between two salt marsh beetle ecotypes: evidence for ongoing speciation. – *Evolution* 61: 184–193.
- Dockx, C. 2007. Directional and stabilizing selection on wing size and shape in migrant and resident monarch butterflies, *Danaus plexippus* (L.), in Cuba. – *Biol. J. Linn. Soc.* 92: 605–616.
- Eckert, C. G. et al. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. – *Mol. Ecol.* 17: 1170–1188.
- Eliasson, C. 2005a. *Coenonympha arcania* pärlgräsfjäril. Nationalnyckeln till Sveriges flora och fauna. Fjärilar: dagfjärilar. Hesperidae-Nymphalidae. – Artdatabanken, SLU, pp. 331–332.
- Eliasson, C. 2005b. *Coenonympha hero* brun gräsfjäril. Nationalnyckeln till Sveriges flora och fauna. Fjärilar: dagfjärilar. Hesperidae-Nymphalidae. – Artdatabanken, SLU, pp. 334–335.
- Eliasson, C. et al. 2005. Nationalnyckeln till Sveriges flora och fauna. Fjärilar: dagfjärilar. Hesperidae-Nymphalidae. – Artdatabanken, SLU.
- Fischer, K. and Fiedler, K. 2002. Reaction norms for age and size at maturity in response to temperature: a test of the compound interest hypothesis. – *Evol. Ecol.* 16: 333–349.
- Fric, Z. and Konvička, M. 2002. Generations of the polyphenic butterfly *Araschnia levana* differ in body design. – *Evol. Ecol. Res.* 4: 1017–1032.
- Gapare, W. J. et al. 2005. Genetic diversity of core and peripheral Sitka spruce (*Picea sitchensis* (Bong.) Carr) populations: implications for conservation of widespread species. – *Biol. Conserv.* 123: 113–123.
- Gärdenfors, U. (ed.) 2005. The 2005 Red List of Swedish species. – Swedish Species Information Centre, Swedish Univ. of Agricultural Sciences, Uppsala, Sweden.
- Gilchrist, A. S. et al. 2000. Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. – *Evol. Dev.* 2: 114–124.
- Haynes, K. and Cronin, J. 2006. Interpatch movement and edge effects: the role of behavioral responses to the landscape matrix. – *Oikos* 113: 43–54.
- Heino, M. and Hanski, I. 2001. Evolution of migration rate in a spatially realistic metapopulation model. – *Am. Nat.* 157: 495–511.
- Helgadóttir, Á. and Snaydon, R. W. 1986. Patterns of genetic variation among populations of *Poa pratensis* L. and *Poa capillaris* L. from Britain and Iceland. – *J. Appl. Ecol.* 23: 703–719.
- Higgins, L. G. and Kiley, N. D. 1993. *Butterflies of Britain and Europe*, reprint der 5. Aufl. – Harper Collins.
- Hill, J. K. et al. 1999. Evolution of flight morphology in a butterfly that has recently expanded its geographic range. – *Oecologia* 121: 165–170.
- Hoffmann, A. A. and Blows, M. W. 1994. Species borders: ecological and evolutionary perspectives. – *Trends Ecol. Evol.* 9: 223–227.
- Ivinskis, P. 2004. *Lepidoptera of Lithuania*. – Annotated catalogue.
- Jones, L. et al. 1968. The effects of population size and selection intensity in selection for a quantitative character in *Drosophila* II. Long-term response. – *Genet. Res.* 12: 249–266.
- Kadlec, T. and Vrba, P. 2007. A second generation of *Coenonympha arcania* (Linnaeus, 1761) in northern Bohemia, Czech Republic (Lepidoptera, Satyriinae). – *Atalanta* 38: 181–183.
- Kesküla, T. 1992. Distribution maps of Estonian butterflies (Lepidoptera: Hesperioidea, Papilionoidea). – *Acta Musei Zoologici Univ. Tartuensis*.
- Kirkpatrick, M. and Barton, N. H. 1997. Evolution of a species' range. – *Am. Nat.* 150: 1–23.
- Kölliker-Ott, U. M. et al. 2004. Field dispersal and host location of *Trichogramma brassicae* is influenced by wing size but not wing shape. – *Biol. Control* 31: 1–10.

- Kölliker-Ott, U. M. et al. 2003. Are wing size, wing shape and asymmetry related to field fitness of *Trichogramma* egg parasitoids? – *Oikos* 100: 563–573.
- Kudrna, O. 2002. The distribution atlas of European butterflies. – *Oedipus* 20: 1–342.
- Lesica, P. and Allendorf, F. W. 1995. When are peripheral populations valuable for conservation? – *Conserv. Biol.* 9: 753–760.
- Levin, D. 1970. Developmental instability and evolution in peripheral isolates. – *Am. Nat.* 104: 343–353.
- Littell, R. C. et al. 1996. SAS system for mixed models. – SAS Inst., Cary.
- Littell, R. C. 2002. SAS for linear models, 4th ed. – SAS Inst., Cary.
- Mayr, E. 1954. Change of genetic environment and evolution. – In: Huxley, J. et al. (eds), *Evolution as a process*. Allen and Unwin, pp. 157–180.
- Merckx, T. and Van Dyck, H. 2006. Landscape structure and phenotypic plasticity in flight morphology in the butterfly *Pararge aegeria*. – *Oikos* 113: 226–232.
- Nilsson, N.-E. (ed.) 1990. National atlas of Sweden. The forest. – Skogsstyrelsen, in Swedish.
- Nylin, S. and Svärd, L. 1991. Latitudinal patterns in the size of European butterflies. – *Holarct. Ecol.* 14: 192–202.
- Palang, H. et al. 1998. Landscape diversity changes in Estonia. – *Landscape Urban Plann.* 41: 163–169.
- Palmer, A. R. 1994. Fluctuating asymmetry analyses: a primer. – In: Markov, T. A. (ed.), *Developmental instability: its origins and evolutionary implications*. Kluwer, pp. 335–364.
- Ricketts, T. H. 2001. The matrix matters: effective isolation in fragmented landscapes. – *Am. Nat.* 158: 87–99.
- Roff, D. A. 1997. *Evolutionary quantitative genetics*. – Chapman and Hall.
- Roff, D. and Fairbairn, D. 2001. The genetic basis of dispersal and migration, and its consequences for the evolution of correlated traits. – In: Clobert, J. et al. (eds), *Dispersal*. Oxford Univ. Press, pp. 191–202.
- Saastamoinen, M. 2007. Heritability of dispersal rate and other life history traits in the Glanville fritillary butterfly. – *Heredity* 100: 39–46.
- Saccheri, I. J. et al. 2001. Effects of bottlenecks on quantitative genetic variation in the butterfly *Bicyclus anynana*. – *Genet. Res.* 77: 167–181.
- Santamaria, L. et al. 2003. Plant performance across latitude: the role of plasticity and local adaptation in an aquatic plant. – *Ecology* 84: 2454–2461.
- Schtickzelle, N. et al. 2006. Dispersal depression with habitat fragmentation in the bog fritillary butterfly. – *Ecology* 87: 1057–1065.
- Schultz, C. and Crone, E. 2001. Edge-mediated dispersal behavior in a prairie butterfly. – *Ecology* 82: 1879–1892.
- Stamps, J. A. et al. 1987. The effects of edge permeability and habitat geometry on emigration from patches of habitat. – *Am. Nat.* 129: 533.
- Sulcs, A. and Viidalepp, J. 1974. Verbreitung der Grossschmetterlinge im Baltikum (Tagfalter, Diurna). – *Deutsch. Ent. Z.* 21: 353–403.
- Talloe, W. et al. 2008. Environmental stress and quantitative genetic variation in butterfly wing characteristics. – *Evol. Ecol.*, doi: 10.1007/s10682-008-9246-4.
- Taylor, P. and Merriam, G. 1995. Wing morphology of a forest damselfly is related to landscape structure. – *Oikos* 73: 43–48.
- Thomas, C. D. et al. 1998. Evolutionary consequences of habitat fragmentation in a localized butterfly. – *J. Anim. Ecol.* 67: 485–497.
- Tolman, T. and Lewington, R. 1998. *Die Tagfalter Europas und Nordwestafrikas*. – Franckh-Kosmos Verlag.
- Travis, J. and Dytham, C. 1998. The evolution of dispersal in a metapopulation: a spatially explicit, individual-based model. – *Proc. R. Soc. B* 265: 17–23.
- van Buskirk, J. and Willi, Y. 2006. The change in quantitative genetic variation with inbreeding. – *Evolution* 60: 2428–2434.
- Van Dyck, H. and Wiklund, C. 2002. Seasonal butterfly design: morphological plasticity among three developmental pathways relative to sex, flight and thermoregulation. – *J. Evol. Biol.* 15: 216–225.
- Van Rossum, F. et al. 2003. A comparative study of allozyme variation of peripheral and central populations of *Silene nutans* L. (Caryophyllaceae) from western Europe: implications for conservation. – *Plant Syst. Evol.* 242: 49–61.
- van Swaay, C. and Warren, M. 1999. *Red data book of European butterflies (Rhopalocera)*. Nature and environment. – Council of Europe Publishing.
- Viidalepp, J. 1995. *Eesti Suurliblikate Nimestik (Catalogus Macrolepidopterorum Estoniae)*. – Teaduste Akadeemia Kirjastus.
- Warren, M. S. et al. 2001. Rapid responses of British butterflies to opposing forces of climate and habitat change. – *Nature* 414: 65–69.
- Wickman, P.-O. 1992. Sexual selection and butterfly design – a comparative study. – *Evolution* 46: 1525–1536.
- Wilson, J. B. et al. 1991. A test of the low marginal variance (LMV) theory, in *Leptospermum scoparium* (Myrtaceae). – *Evolution* 45: 780–784.
- Windig, J. J. 1991. Quantification of Lepidoptera wing patterns using an image analyzer. – *J. Res. Lepidoptera* 30: 82–94.
- Windig, J. J. et al. 2000. How useful is fluctuating asymmetry in conservation biology: asymmetry in rare and abundant *Coenonympha* butterflies. – *J. Insect Conserv.* 4: 253–261.