Asymmetry patterns across the distribution range: does the species matter?

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An important question in evolutionary ecology is whether different populations across a species range, from core to periphery, experience different levels of stress. The estimation of developmental instability has been proposed as a useful tool for quantifying the degree of environmental and genetic stress that individuals experience during their development. Fluctuating asymmetry, the unsigned difference between the two sides of a bilaterally symmetrical trait, has been suggested to reflect the levels of developmental instability in a population. As such, it has been proposed as a useful tool for estimating changes in developmental instability and in stress response in populations across a range of environmental conditions. Recent studies focusing mostly on birds have detected increasing fluctuating asymmetry from core to periphery across the distribution range, suggesting that peripheral populations may experience higher levels of environmental and/or genetic stress. Most of these comparisons were done for single taxa across a single gradient. However, different species are predicted to respond differently to environmental shifts across the range. We compared asymmetry patterns in wing morphology in populations of two *Euchloe* butterfly species across their opposing ranges in Israel. Contrary to the patterns observed in birds across the same gradient, bilateral asymmetry did not increase or shift towards the periphery in either of the butterfly species. If fluctuating asymmetry in these traits reflects levels of stress, these results may partly reflect the fact that the range of these two butterfly species is limited by the distribution of their host plant, rather than by abiotic environmental variables. In addition, developing pierids can diapause during harsh seasons and can persist in resource-rich patches, thus minimizing the environmental stress perceived by developing individuals. We conclude that accounting for differences in species' life histories and range-limiting factors is necessary in order to better predict patterns of developmental instability across spatial and environmental gradients. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **81**, 313–324.

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INTRODUCTION

As one moves from the interior towards the periphery of a species's range, the spatial distribution, dynamics and structure of populations change. Populations often become more patchy, isolated and transient and their probability of extinction increases towards the edge of the range (Carter & Prince, 1981; Maurer, 1994; Brown, Stevens & Kaufman, 1996). In addition, genetic diversity often declines at the periphery, where local populations face bottlenecks, genetic drift and stochastic processes (reviewed in Hoffmann & Blows, 1994; Hoffmann & Parsons, 1991). Beyond the edge of the range, viable populations cannot persist over time. However, it is presently unclear to what extent those individuals that manage to persist at the very edge of the range actually experience higher lev-

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els of environmental and/or genetic stress at a given point in time as compared with those in the more interior areas of the range, and whether the patterns are consistent in species with different life histories. Individuals that manage to persist at the edge of the range may either be adapted to marginal environments or select relatively favourable resource-rich habitat patches and periods in which developmental conditions are comparable to those experienced in more interior areas of the range. Unveiling whether or not individuals from populations located near the periphery of the range experience high genetic and environmental stress is important for better understanding the dynamics of range limits and in predicting population persistence in the face of environmental and climatic change.

The estimation of developmental instability has been proposed as a useful tool for quantifying the degree of environmental and genetic stress that individuals experience during their development. Developmental instability has been estimated using various tools, most notably fluctuating asymmetry (FA; Van Valen, 1962; Leary & Allendorf, 1989; Palmer, 1994). Fluctuating asymmetry is the unsigned difference between the two sides of a bilaterally symmetrical morphological trait (Ludwig, 1932). The development of both sides of a bilaterally symmetrical trait is presumably under the influence of a single set of genes (Clarke, 1993; Markow, 1995; Møller & Swaddle, 1997). Therefore, differences between the two sides of the body are thought to represent variation of environmental origin (Palmer & Strobeck, 1986; Clarke, 1993). The findings of studies examining whether such asymmetry may also reflect genetic stress (e.g. resulting from low genetic diversity) have been mixed (Bjorksten, Fowler & Pomiankowski, 2000b; Hochwender & Fritz, 1999; Lu & Bernatchez, 1999; Waldmann, 1999; Lens *et al*., 2000; Kark *et al*., 2001).

Two other major forms of asymmetry are discussed in the literature, as described by the statistical distribution of the right minus the left sides of a trait in individuals within a population. These are (i) directional asymmetry (DA), in which one side of the body is consistently larger than the other, showing a skewed distribution of the asymmetry estimate in the population, and (ii) antisymmetry, where most individuals deviate from symmetry towards either the right or left sides, with the population showing a bimodal or platykurtic distribution (Leary & Allendorf, 1989). DA and antisymmetry have not been considered reliable indicators of developmental instability due to their unknown heritable component (Van Valen, 1962; Palmer & Strobeck, 1986; Leary & Allendorf, 1989). Yet increasing evidence suggests that a continuum exists between the different types of asym-

metry and that DA and antisymmetry, in addition to FA, may often reflect the inability to carry out identical development of bilateral traits in the face of stress (Graham, Freeman & Emlen, 1993, 1998; Kark, 2001; Lens *et al*., 2002).

Several recent studies on birds and mammals have found increasing fluctuating asymmetry towards the periphery of the distribution range (Møller, 1995; Carbonell & Tellería, 1998; Auffray *et al*., 1999). Changes in bilateral asymmetry across a steep environmental gradient from Mediterranean core areas to the arid periphery have been examined in 11 chukar partridge (*Alectoris chukar*) populations in Israel (Kark, 2001). It was found that in addition to a substantial increase in bilateral asymmetry, the expression of the directional asymmetry component significantly increased along the range such that the distribution shifted from FA to DA in the ecotone region and towards the periphery of the range (Kark, 2001). An important question that arises is whether these results are expected to be general and to apply to species with different life histories, distribution ranges and range-limiting factors from those of the birds. This question may have substantial implications for better understanding of the differences in species' responses across the distribution range.

When compared across their range, different species are expected to differ in the patterns of stress that they experience depending on their physiological, behavioural and life-history traits, which directly influence the size, grain, variation, and spatial scale of the environment they perceive. For instance, during development, individuals of species that move around relatively large areas may experience higher spatial variability than do more sedentary species that sample the same environment more locally (e.g. an insect that is confined during its development to one single branch). Those individuals that are able to move from one location to another may be able to avoid harsh periods and environments by moving to more favourable regions or patches. While some species can avoid more stressful seasons, periods or years (e.g. a migratory species, an insect that diapauses for several years, or an annual plant that can remain dormant as a seed), other species may respond more clearly to environmental shifts (e.g. perennial species that need to cope with temporal shifts in environmental conditions year after year).

In this study, we aimed to investigate spatial patterns in bilateral asymmetry in a pierid butterfly species (*Euchloe belemia*) that shows a very similar distribution pattern to the chukar partridge (*A. chukar*) along the ecogeographical gradient in Israel. The species reaches the edge of its distribution range in the same region. However, in contrast to *A. chukar*, during its development an individual of

E. belemia is limited to a very restricted local environment of a single host plant. Hence, its range is more likely to be restricted by the range of its host plants, rather than by environmental stresses that work directly on the animals themselves (Benyamini, 2002). In addition, unlike *A. chukar*, it has the potential to avoid more stressful periods by remaining dormant (in diapause; Benyamini, 2002). Based on this set of life-history characteristics we predicted that *Euchloe* populations across the range, unlike *A. chukar*, would not show substantial differences in developmental instability. We also studied a second closely related species (*E. falloui*) with very similar host plant preference and similar morphology, ability to diapause, and many other life-history traits (Benyamini, 2002), yet with an opposing distribution range: the Mediterranean-arid ecotone of Israel is at the northernmost, rather than the southernmost, periphery of its range (Fig. 1).
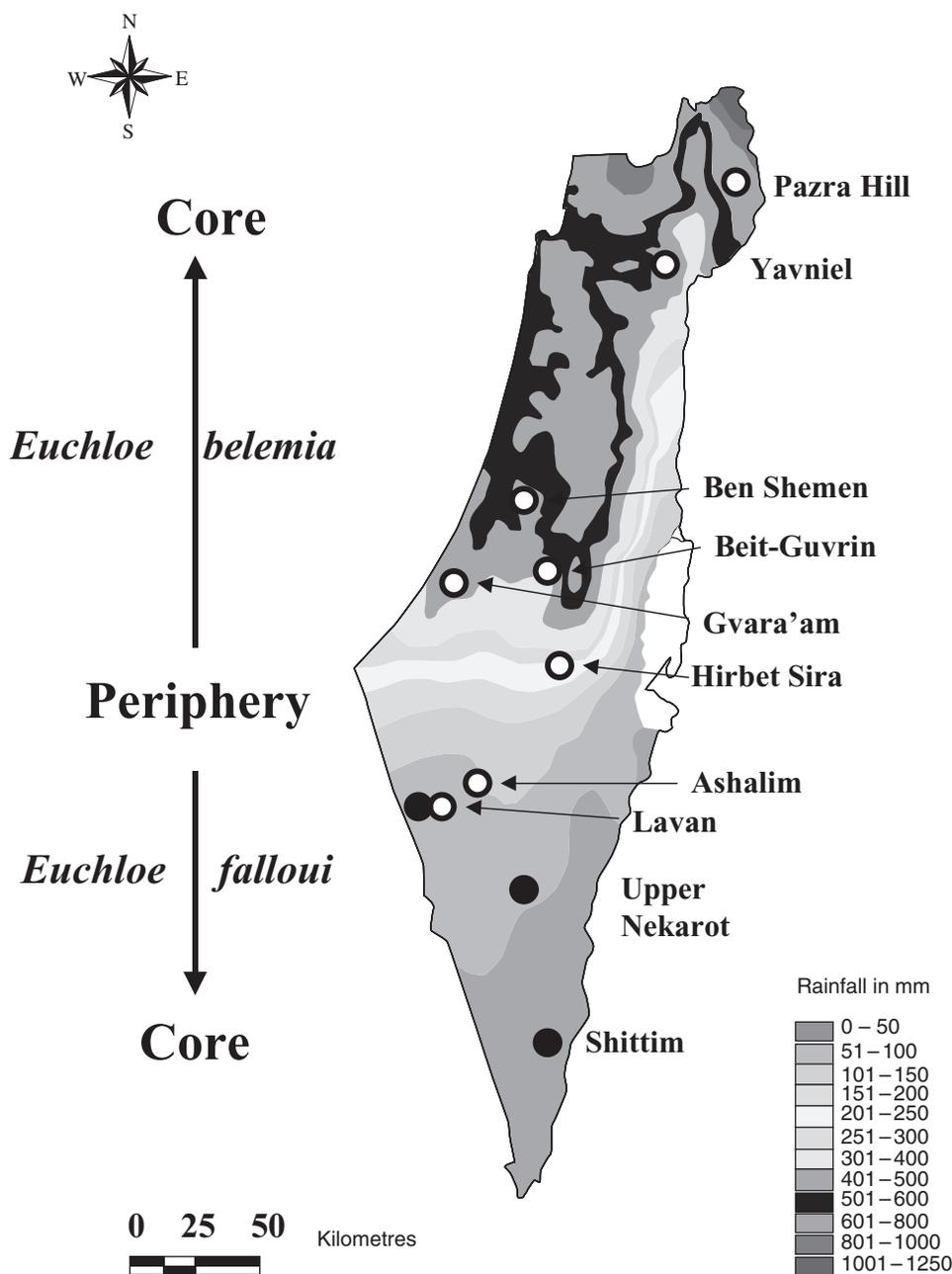
Figure 1. Study area and populations of *Euchloe belemia* (◐) and *Euchloe falloui* (●) sampled along a rainfall gradient (data from the Hebrew University of Jerusalem GIS Center). One individual of *E. falloui* was surveyed in Ashalim and two *E. belemia* individuals in Upper Nekarot (not marked).

MATERIAL AND METHODS

STUDY AREA

A steep climatic and environmental gradient occurs across short geographical distances in Israel, from mesic Mediterranean areas in the north and centre to more arid areas in the Negev desert (Bitan & Rubin, 1991). While annual rainfall in the northern and central parts of the study region averages over 500 mm and may exceed 1000 mm, the mean annual rainfall rapidly drops to less than 50 mm only several dozen kilometres away in the arid regions. A particularly steep part of the rainfall gradient occurs in the northern Negev ecotone between Mediterranean and desert ecosystems, where within a relatively narrow belt of less than 60 km, the mean annual rainfall decreases from 450 mm to 150 mm and its spatiotemporal variability increases (Fig. 1; Bitan & Rubin, 1991, and the Israel Meteorological Service). This climatic cline is associated with a change in phytogeography from largely Mediterranean to largely Saharo-Arabian vegetation, with a combination of both in the northern Negev ecotone region (Danin & Plitman, 1987). Along this cline, many Mediterranean, Saharo-Arabian and Asian species reach the edge of their continuous southern, northern, and western distribution ranges, respectively (Kadmon & Danin, 1997; Benyamini, 2002).

THE SPECIES

Euchloe distribution
Two pierid species, *E. belemia* and *E. falloui*, were selected for the study. These species are morphologically very similar, yet *E. falloui* lacks the white spot within the black discal spot that is found in *E. belemia* (Benyamini, 2002). *E. belemia* is distributed in northern and central Israel and reaches the periphery of its range in the Negev desert. *E. falloui* is a North African and Saharan species and reaches the edge of its range in the northern Negev ecotone, this being the northern periphery of its range (as opposed to the southern periphery for *E. belemia*). The two species are sympatric in the central Negev Highlands (Benyamini, 2002).

Life history
Host plants: In Israel, larvae of both species are restricted to Brassicaceae host plants, *E. belemia* showing a wider range of larval host species compared with *E. falloui*. The latter species has been recorded on *Moricandia nitens*, *Moricandia sinaica*, *Zilla spinosa*, *Diplotaxis acris*, *Schouwia thebaia*, *Hirschfeldia incana* and *Reseda* sp. The edge of the *Moricandia* distribution range also marks the edge of the *E. falloui* distribution range. *E. belemia* has been reported feeding on: *Hirschfeldia incana*, *Isatis lusitanica*, *Neslia*

apiculata, *Ochthodium aegyptiacum*, *Moricandia nitens*, *Sisymbrium irio*, *Erucaria rostrata*, *Erucaria hispanica*, *Rapistrum rugosum*, *Sinapis arvensis* and *Diplotaxis harra*. In the Lavan region (Fig. 1), the two species are sympatric and both feed on *Moricandia nitens*. *Diplotaxis* sp. may be used but they are not very common, being near their northern distribution margins (D. Benyamini, pers. comm.).

Diapause: Both species, but especially *E. falloui,* have the potential to diapause for exceptionally long periods as pupae to avoid dry years and seasons (Benyamini, 2002). Only a certain proportion (25–30%) of the pupae hatch every year pending rainfall. Egg and larval diapause of over 7 years has been recorded in the region in *E. falloui* (Benyamini, 2002). Diapause of *E. belemia* is somewhat shorter – up to 7 years has been observed – and is restricted to the pupal stage (Benyamini, 2002). The two species exhibit multiple generations per season, usually two to three, with the second- and third-generation individuals showing increasingly paler green stripes (and discal spots) on the white background.

THE STUDY POPULATIONS

Fieldwork was conducted during the spring of 1999 (March–April). All fieldwork was done during the same season to minimize temporal variation. We sampled butterflies along the steep rainfall gradient, from areas with mean annual rainfall of over 700 mm in the northern Golan Heights, at Pazra Hill, to areas with a mean annual rainfall of *c*. 30 mm in the southern Negev, at Shittim (Fig. 1). The distance between these two extremes of the sampled range is less than 400 km. Eight populations of *E. belemia* and three of *E. falloui* were studied across their distribution range (Table 1). Single individuals were found for each of the species in one additional location (Table 1; Fig. 1), marking the most marginal individuals detected during the study season. Populations were sampled across their distribution range from the periphery towards the core in an area of approximately 1 km^2 during 1 or 2 days. Because the species shows a patchy distribution in Israel, especially in the southern regions, where the host plant distribution is patchy, although we did not have genetic information on the species, we could define populations based on area size. In all cases, sample size for males was higher than for females, probably due to the fact that females were more difficult to locate.

The sex of the butterflies was determined visually in the field upon capture, with later verification of sex during wing removal in the laboratory. Sex determination was based on visual examination of posterior abdominal structures and observation of the presence of claspers in males. Butterflies were stored

Table 1. Location and sample characteristics of *Euchloe belemia* and *E. falloui* populations studied in Israel, arranged from north to south

Location	Species	N	Relative density	Weather conditions	Ecogeographical region
Pazra Hill	*E. belemia*	19	Medium +	~27°, clear, mild wind	Mediterranean
Yavniel	*E. belemia*	33	Medium*	~20°, mostly clear, mild wind	Mediterranean
Ben Shemen	*E. belemia*	34	Medium-high	~23–27°, few clouds, mild wind	Mediterranean
Beit Guvrin	*E. belemia*	25	High*	~22°, clear, medium wind	Ecotone
Gvara'am	*E. belemia*	30	Very high	~27°, few clouds, mild wind	Ecotone
Hirbet Sira	*E. belemia*	32	Low +	~27°, few clouds to cloudy, mild to strong wind	Ecotone
Ashalim	*E. belemia*	14	Medium–high	~20–26°, clear, medium wind	Arid
Lavan	*E. belemia*	24	Medium–high	~22–24°, mostly clear, mild wind	Arid
Upper Nekarot	*E. belemia*	16	Very low	~20–26°, clear, medium wind	Arid
Ashalim	*E. falloui*	14	Very low	~22°	Arid
Lavan	*E. falloui*	38	Medium–high	~22–24°, mostly clear, medium wind	Arid
Upper Nekarot	*E. falloui*	16	Medium–low	~20–26°, clear, medium wind	Arid
Shittim (Upper Hayun)	*E. falloui*	14	Low	~22°, clear with some clouds, medium–high wind	Arid

*Estimated retrospectively based on sampling effort. Estimated butterfly densities were relative to other populations; these and weather conditions were noted at the time of sampling. Temperature was taken in the shade. Ecogeographical regions were pre-determined; ecotone indicates the Mediterranean-desert ecotone region. See Fig. 1 for locations.

in separate envelopes and placed in a −20° or −80° freezer upon arrival back in the laboratory (between 6 h and 34 h after capture). Wings were removed from the bodies before measuring commenced by holding tweezers at the base of the joint and pulling away from the thorax. Sex was verified at this step. Each individual's wings were replaced into the individual envelopes.

TRAIT MEASUREMENTS

For *E. belemia,* five vein and two discal spot measurements were taken. Vein measurements are shown in Figure 2. Discal spot measurements included: (i) the total area of the discal spot (SA) and (ii) the area of the white portion in the inner part of the black discal spot alone (WS). For *E. falloui,* the same five vein measurements and one spot measurement (SA) were taken (in this species there is no white area within the dark discal spot and therefore only the total area of the dark discal spot was measured; see Nijhout (1991) for further details on discal spot).

Distances between veins were measured using NIH *Image* software Version 1.56 and a Power Macintosh computer connected to a Panasonic video monitor and a Panasonic Industrial Colour GP-US502E video camera with a Sigma Macro ø 52 1 : 28 lens. The height of the camera was fixed and we controlled for light in the room to reduce measurement error using a Kaiser lighting system. The area of each discal wing spot was calculated using the NIH *Image* tool at a threshold, which was determined in each case to optimize outline clarity and reduce fragmentation. For *E. belemia* the area of the white spot was also measured. For video imaging of the veins, wings were placed on a glass microscope slide (72 × 26 mm) wet with 100% ethanol. The ethanol served both to lay the wing flat against the glass slide (eliminating the need for a cover slide) and to make the wing highly transparent, optimizing vein visibility. For discal spot measurements, wings were placed dry between two glass microscope slides, the weight of the top slide being sufficient to hold them flat. Wings were not wet with ethanol as this reduced clarity of the discal spots. After measuring each set of wings they were placed back in the envelope until the measurements were repeated. The same person (E.S.) performed all measurements, over a total period of 3 weeks. All individuals were first measured for all traits, with individuals of both species and all populations drawn in random order. This was done to avoid introducing confounding trends related to changes in measurement error with increasing experience. After all individuals had been measured once, the process was repeated.

STATISTICAL ANALYSES

Unbiased estimation of fluctuating asymmetry
Levels of asymmetry are often small and measurement error can bias estimates upward if not corrected

A

B
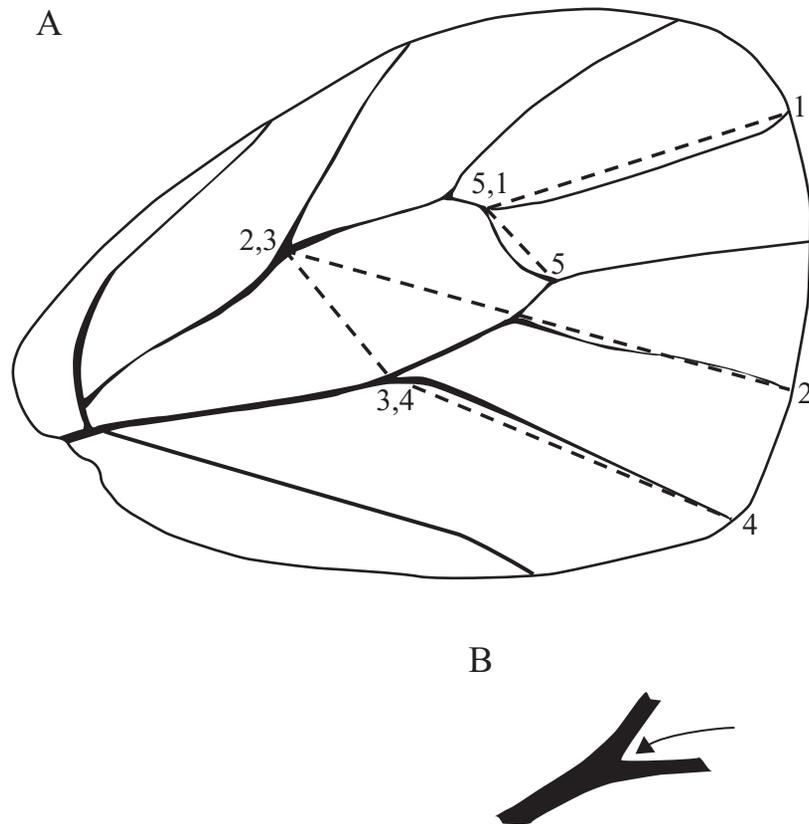
Figure 2. Vein distances measured in the upper wing of *Euchloe belemia* and *E. falloui* (A). Measurements were taken as the shortest distance between each pair of nodes (dashed lines) for five traits: (1) top vein; (2) wing width; (3) cell width; (4) bottom vein; (5) cell end. Measurements were taken from the inner node of vein bifurcation (B).

for properly (Palmer & Strobeck, 1986). Therefore, mixed model analysis of repeated measurements on both sides are required to obtain unbiased estimates of population and individual FA levels (van Delden, 1988; Van Dongen, Molenberghs & Matthysen, 1999; Van Dongen, 2000). Furthermore, patterns of FA may be confounded with other types of asymmetry, namely DA and antisymmetry, for which the link with developmental stability is less clear (Graham *et al.*, 1993; Palmer & Strobeck, 1986; Palmer & Strobeck, 1992; Palmer, 1999). We applied mixed regression analysis (Van Dongen *et al.*, 1999) to test for directional asymmetry, to evaluate the magnitude of the measurement error relative to the level of fluctuating asymmetry, and to obtain unbiased estimates of individual asymmetry. Whereas FA and antisymmetry cannot be separated statistically with high power, they can be distinguished reliably through visual inspection of scatter plots (see Rowe, Repasky & Palmer (1997) and Lens & van Dongen (1999) for comparable approaches). Data were inspected for outliers that were likely to reflect developmental errors with a different basis from those with true FA.

Trends in asymmetry across a distribution range
To test the hypothesis that asymmetry increases along the distribution range towards the periphery, we tested for an increasing or decreasing trend in levels of FA in populations across the distribution range of both species in a linear mixed model. Individual, trait-specific estimates of asymmetry (after taking the absolute value, i.e. the unsigned asymmetry) obtained from the mixed regression analyses (described above) were used as the dependent variable. Location, taken as the sequential number of each population ordered from north to south (core–periphery for *E. belemia* and periphery–core for *E. falloui*, Fig. 1), species and location × species interaction were treated as fixed-effect explanatory variables. In this way a regression line was estimated for each species separately. These two regression lines were estimated across all traits. Between-trait variation in this association was incorporated into the model by treating trait and the trait–location interaction as random effects. Because between-trait variation may be different among *E. belemia* and *E. falloui*, variance components were

estimated and tested for each of these two species separately.

Between-population variation in asymmetry values around the regression line was incorporated into the model using population and the population–trait interaction as random effects. Variance components were estimated and tested for each species separately. By incorporating this population effect into the model, each population contributed only one degree of freedom to the tests of the fixed effects. Failing to do this may lead to an inflated number of degrees of freedom since each individual would contribute one degree of freedom. Tests of fixed effects were performed by traditional F-tests approximating the denominator degrees of freedom by the procedure of Satterthwaites. Significance of variance components was quantified by one-tailed likelihood ratio tests (details in Verbeke & Molenberghs, 2000).

Asymmetry values of the different traits in individuals were analysed in a multivariate analysis framework (i.e. by treating traits as repeated measures in a mixed regression model). Such a routine generally produces results comparable to those obtained with composite indices of FA. However, because the degrees of freedom in multivariate models reflect the number of individuals rather than the number of traits by individuals, pseudoreplication is avoided (Lens *et al.*,

2002). Between-trait correlations were modelled by assuming an unstructured design of the variance-covariance matrix of the residual values. The variance-covariance matrix was estimated for each of the two species separately.

RESULTS

Table 2 summarizes the results of the mixed regression analyses for the two species and the seven traits measured. The relative amount of measurement error varied considerably between traits and species, and was relatively larger for *E. falloui*. One trait (cell width) showed significant DA in both species. We incorporated all traits into the following analysis since the obtained unbiased estimates of individual- and trait-specific asymmetry were corrected for measurement error and DA. Visual inspection of the distribution of the signed asymmetry values obtained after correction for measurement error and DA did not show indications of the presence of antisymmetry (data not shown). Since in some cases there were outlying observations, unsigned asymmetry values were log transformed to reduce the weight that these observations could have in the parameter estimates.

Significance tests and effect sizes of the linear mixed model are summarized in Table 3. Results

Table 2. Tests of significance of levels of fluctuating asymmetry (FA; likelihood ratio test) and the presence of directional asymmetry (DA; F-test with degrees of freedom approximated according to Satterthwaites' procedure; see Methods) in measured traits of *Euchloe falloui* and *E. belemia* as obtained from mixed-effects regression models

	E. falloui				E. belemia			
	FA			DA	FA			DA
Trait	Ind	FA	ME		Ind	FA	ME	
BV	$\chi^2_1 = 2$ 0.29	0.014	0.036	$F_{1,53} = 0.67$	$\chi^2_1 = 3.8^*$ 0.67	0.014	0.05	$F_{1,194} = 0.62$
CE	$\chi^2_1 = 1.3$ 0.035	0.007	0.025	$F_{1,55} = 0.95$	$\chi^2_1 = 541^{***}$ 0.14	0.11	0.002	$F_{1,189} = 3.69$
CW	$\chi^2_1 = 12.0^{***}$ 0.081	0.002	0.002	$F_{1,61} = 8.6^{**}$	$\chi^2_1 = 142^{***}$ 0.10	0.004	0.001	$F_{1,207} = 14.7^{***}$
SA	$\chi^2_1 = 14.6^{***}$ 2.49	0.030	0.003	$F_{1,65} = 0.0$	$\chi^2_1 = 348^{***}$ 2.62	0.04	0.006	$F_{1,208} = 2.53$
TV	$\chi^2_1 = 0.0$ 0.19	0.016	0.23	$F_{1,51} = 1.1$	$\chi^2_1 = 953^{***}$ 0.57	0.18	0.003	$F_{1,180} = 1.16$
WW	$\chi^2_1 = 1.8$ 0.54	0.14	0.38	$F_{1,54} = 0.87$	$\chi^2_1 = 153^{***}$ 0.94	0.012	0.009	$F_{1,199} = 1.34$
WS	—			—	$\chi^2_1 = 53^{***}$ 0.032	0.002	0.001	$F_{1,203} = 3.86$

Significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Traits: BV = bottom vein; CE = cell end; CW = cell width; SA = black spot area, TV = top vein; WW = wing width; WS = white spot area. Under each test of FA, variance components of the mixed-effects regression model are given (between-individual variation in size (Ind), variation in individual slopes (FA) and degree of measurement error (ME)).

Table 3. Overview of significance tests of fixed and random effects of the linear mixed model analysing variation in individual trait-specific asymmetry values in two species of *Euchloe*

Source	Effect	Significance test
Fixed effects		
Location	–	$F_{1,3} = 1.86$, $P = 0.26$
Species	–	$F_{1,9} = 0.08$, $P = 0.78$
Location X species	–	$F_{1,3} = 2.59$, $P = 0.20$
Random effects		
Trait (*E. belemia*)	0.52*	$\chi^2_1 = 30.5$, $P < 0.0001$
Trait (*E. falloui*)	0.19*	$\chi^2_1 = 30.7$, $P < 0.0001$
Trait X location (*E. belemia*)	0.02	$\chi^2_1 = 2.1$, $P = 0.14$
Trait X location (*E. falloui*)	0.30*	$\chi^2_1 = 33.0$, $P < 0.0001$
Population (*E. belemia*)	0.07	$\chi^2_1 = 1.4$, $P = 0.24$
Population (*E. falloui*)	0.10*	$\chi^2_1 = 4.2$, $P = 0.04$
Population X trait (*E. belemia*)	0.54*	$\chi^2_1 = 207.2$, $P < 0.0001$
Population X trait (*E. falloui*)	0.00	$\chi^2_1 = 0$, $P = 1$

Significance: *$P < 0.05$. *F*-test or likelihood ratio test was performed.

suggest no association between average asymmetry and the relative location of the population across the distribution range in either of the two study species (there were no significant *F*-tests of fixed effects). In addition to the highly significant between-trait variation in average asymmetry seen in both species (significant random trait effect; Table 3) there was a significant trait X location random effect for *E. falloui*. Thus while some traits did show an association with location, others did not. Trait-by-trait analysis showed an association for discal spot area (positive association: slope = 0.71 (±0.27), $t_7 = 2.61$, $P = 0.03$) and for top vein (negative association: slope = –0.87 (±0.29), $t_8 = –3.04$, $P = 0.02$). It should be noted that these associations did not remain statistically significant after Bonferroni correction. Therefore, both associations should be viewed as the extremes of a distribution. However, since they were of comparable magnitude but opposite in sign, this analysis clearly shows that patterns observed at different traits may be completely different. Between-population variation in asymmetry across all traits (as estimated by the random population effect, Table 2) was relatively low in both species and was significant only in *E. falloui*. For the Mediterranean species, *E. belemia,* there was a strong and highly significant population–trait interaction, indicating that populations do differ in their asymmetry values for this species but that the patterns of between-population variation vary among traits. Inspection of this trait-specificity for cell width, the trait showing significant DA, did not reveal that it was responsible for the between-trait variation. Moreover, the associ-

ation with location in *E. falloui* indicates the absence of a population asymmetry parameter.

DISCUSSION

A suite of factors that may vary among species limits their distribution ranges. Accordingly, individuals of different species may respond to life at the edge of the range in different ways. Their responses, adaptations and the stresses they face may depend on the species characteristics and life histories, as well as on the environmental factors (biotic and abiotic) that limit species distributions. For example, when the range-limiting factor is the availability of a given resource, individuals at the periphery may be restricted to resource-rich patches where the environment is relatively favourable. If the size of a patch is sufficiently large to sustain a viable population, we do not predict that individuals occupying such patches at the edge of the range will be more stressed than those in core areas. If indeed FA reflects stress in these species and traits, this may be the case in our study of the two Lepidopteran species, *E. belemia* and *E. falloui* in Israel. Bilateral asymmetry in all wing vein measurements and in discal spot area of the two butterfly species did not increase towards the periphery of the distribution range. In addition, levels of asymmetry did not significantly differ between the two species when compared in sympatric populations located close to their respective range peripheries. As the aim of our study was to compare asymmetry not among sympatric populations of the two species but rather along the range of each species, only one pair of sympatric populations was studied. As such, we cannot judge the generality of

this pattern. At present we do not know of evidence for interbreeding between the two species (D. Benyamini, pers. comm.). Such hybridization may lead to differences among populations across the range (Tebb & Thoday, 1954; Barton & Hewitt, 1982; Graham & Felley, 1985; Leary, Allendorf & Knudsen, 1985; Hochwender & Fritz, 1999; Waldmann, 1999).

These results oppose those found for the phasianid bird species, *A. chukar,* across the same climatic gradient in Israel (Kark, 2001). Indeed, as predicted in the bird study in Israel, levels of developmental instability were shown to be significantly higher in peripheral populations compared with those in the core. Substantial physiological work suggests that *A. chukar* is stressed in hot and arid environments (Frumkin, 1983; Kam, 1986; Carmi-Winkler, Degen & Pinshow, 1987). Individuals of *A. chukar* survive for several (estimated 2–4) years and their chicks move around in search of food and to escape predators very early in life (Alkon, 1974; Alkon, 1979). Thus, during development, chicks potentially perceive a wide range of environmental conditions with high spatial heterogeneity. Developing birds cannot temporally escape harsh and less favourable conditions by skipping seasons, a strategy that has been observed in the butterfly species we studied, in particular in the more arid one (*E. falloui*). Nijhout (1991), in his detailed account of the development of butterfly wing patterns, suggested that the three major processes of wing pattern formation, i.e. the determination of wing shape, wing venation and wing colour pattern, all take place during larval life (Nijhout, 1991, fig. 1.14). During these stages the larvae of the two *Euchloe* species studied are often restricted to a patch consisting of a single or few plants (D. Benyamini, pers. comm.). Thus, the scale at which these butterfly species perceive their environment is very different from that of *A. chukar*. On the scale of the butterflies, environmental variation is much smaller compared with that perceived outside the patch. In years in which rainfall is too low to assure sufficient food resources, the majority of individuals will have the option to remain in diapause in the egg, larval or pupal stage. The study year (1999) was relatively dry and hot. It was difficult to locate populations of the desert species, most of which may have skipped this season in diapause at the pupal stage. Only three populations were actually found for the southern species. It would be interesting to compare patterns of asymmetry across the range in dry vs. more mesic years, as well as in diapaused vs. non-diapaused individuals. We predict that various annual plants in the desert will show similar patterns to those seen in the two *Euchloe* species due to their patchy distribution in resource-rich areas and their possibility to avoid dry years as seeds. Lennon *et al*. (2002) recently provided evidence that several tree species in

Alaska tend to occur in especially favourable sites in geographically peripheral distribution areas. The authors suggest that in these marginal areas, the tree species amplify habitat preferences shown in core areas, thus enabling the species to persist at the margins of the range under more favourable environments.

Levels of developmental instability do not appear to change from core to periphery across the range of these two Lepidopteran species. However, we predict that there may well be cases when changes in asymmetry will be found in Lepidopteran species. For example, levels of bilateral asymmetry will be lower in populations in which the larvae go through their entire life cycle on a single plant (as is the case with the populations studied) compared with cases in which individuals need to forage repeatedly for new plants (in the latter case the environment would be predicted to be more stressful). In addition, individuals that have diapaused for a prolonged period of time, although they have a lowered metabolic rate and repeated temperature stress, might show higher developmental instability and thus higher asymmetry levels due to accumulated water stress (F. Nijhout, pers. comm.). It would be interesting to explore these predictions in future studies.

Because the result of this study is a null one, it may be argued that factors other than those considered in our study may have affected the spatial patterns observed. First, the absence of stress response in the traits studied may result from strong selection for perfect symmetry, these traits being highly important for the performance and survival of the individual. We analysed two types of trait, vein length and discal spot size. Under the above claim, if levels of environmental stress changed across the range, we would expect to find consistent differences in asymmetry patterns across the distribution range between these two sets of traits. However, the differences between the two sets of traits were inconsistent (Table 2). Klingenberg (2003) recently suggested that strong integration across wing compartments occurs in flies and that the wing is a single, fairly homogeneous developmental module. Further work in this direction may help understand the response of insect wing traits and their asymmetry to changes in environment.

We studied two different types of characters, vein distance and length and discal spot size. Different selection patterns act on the development of wing shape and vein length and on discal spot formation and symmetry (Nijhout, 1991). The latter may be predicted to show higher variability, possibly being more strongly related to within- or among-species interactions. We would expect that if levels of environmental stress change dramatically along the distribution range, at least the discal spot would show differences

among populations from different areas; this was not the case in this study. Relative population density based on sampling effort was recorded at the time of sampling, together with environmental conditions at the time. Although this does not give a measure of the overall seasonal density variation or of the environment during development, it does give some indication of the conditions as reflected by butterfly population density in each location at the time of sampling.

It could also be suggested that between-population differences in environmental stress may have remained unmasked because of genetic effects. We did not study patterns of genetic diversity across the distribution range of the two *Euchloe* species, and there may well be differences between populations, especially those located at the edge of the range of both species. However, it is unlikely that the effect of genetic stress on FA would counter that of environmental stress such that it would entirely mask a trend in developmental instability, resulting in no apparent differences between populations in their levels of asymmetry across the range over several traits and in both species. Furthermore, we did find variation in levels of individual asymmetry within populations, indicating that bilateral asymmetry may indeed reflect variation in development.

Finally, bilateral asymmetry may not be a good indicator of environmental or genetic stress. While this has been suggested in some cases for sexually selected traits (Bjorksten *et al*., 2000a), there is substantial evidence in the literature that FA can often reflect levels of environmental, although not necessarily genetic, stress (Britten, 1996; Møller & Swaddle, 1997; Kark *et al*., 2001; Lens *et al*., 2002; Réale & Roff, 2003). It could also be argued that the traits we chose for analysis do not reflect changes in environmental stress, wing vein and discal spot asymmetry being a poor indicator of stress. While we are unable to experimentally separate these possibilities in this study, empirical evidence in the literature suggests that butterfly (and other insect) wings, eyespots and veins may serve as useful indicators of environmental stress, at least in some cases, although evidence is not consistent in all cases (Bjorksten *et al*., 2000a; Nijhout, 1991; Brakefield & Breuker, 1996; Klingenberg, McIntyre & Zaklan, 1998; Windig & Nylin, 1999; Clarke, Yen & McKenzie, 2000; Jenkins & Hoffmann, 2000).

IMPLICATIONS FOR CONSERVATION

Trait bilateral asymmetry, and especially fluctuating asymmetry, has been proposed repeatedly as a useful tool to evaluate environmental stress as perceived by developing individuals and, hence, as a potentially useful tool in conservation (Clarke, 1995; Leary &

Allendorf, 1989). Yet in a recent meta-analysis, asymmetry stress relationships were shown to be highly heterogeneous, even among species of the same taxonomic group (Lens *et al*., 2001). Such differences have generally been assumed to reflect between-study variation in mean levels of ambient stress and between-trait variation in stress sensitivity, or to be related to heterogeneity in the accuracy of asymmetry estimation. Our study shows an additional potentially important source of variation, i.e. different species may avoid or cope with environmental stress in different ways. We suggest that in some cases it may be possible for individuals to persist in a non-stressful microenvironment within what may otherwise be considered to be a more stressful environment. In order to make better predictions about the expected levels of bilateral asymmetry as a function of mode of selection (sexually selected vs. non-sexually selected traits), trait functionality (performance vs. non-performance traits), or physiology (poikilotherms vs. homeotherms) across the range, the particular life history, range-limiting factors and spatial distribution of the species under study should be accounted for properly. Failing to do so may restrict future use of bilateral asymmetry as a stress monitor to comparisons within species or populations, rather than across them.

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