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Commentary

An observation of directional asymmetry in wing spots of two Arctic butterflies (*Colias*, Pieridae)

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1. Introduction

Fluctuating asymmetries (small, random departures from perfect symmetry) are often used as measures of the developmental stability of bilaterally symmetrical traits (Palmer & Strobeck 1992). More recently, fluctuating asymmetry of sexually selected traits has attracted considerable attention (*see* e.g., Møller 1990, Polak & Trivers 1994, Evans *et al.* 1995, Møller & Swaddle 1997). However, as Kraak (1997) cautions, traits that might at first appear to be fluctuating asymmetries may, on closer examination, prove to be directional asymmetries (non-random departures from perfect symmetry).

In spite of many careful investigations of traits for directional asymmetry however, few examples have been found, suggesting that it is relatively uncommon. Here, we study the apparently bilaterally symmetrical spot pattern on the wings of two Arctic *Colias* butterflies (*Colias nastes werdandi* and *Colias hecla sulitelma*). Although the spots might be expected to show fluctuating asymmetry we find that, unusually, they show directional asymmetry.

2. Materials and methods

Butterflies were caught during June and July 1995 and 1996 from 12 sites in Sweden and Norway. All specimens were killed by freezing and then placed in absolute ethanol for preservation, after first removing the wings. The wings of each individual were kept in separate entomological collecting envelopes.

Typically *Colias* species have a small oval-shaped spot situated at the distal edge of the discal area between v4 and v6 on the dorsal surface of each forewing. Although usually oval, the spots can vary from round to a thin line. To overcome this, the length and breadth (at the widest part) of each spot was measured. Multiplying these two figures together gave a very approximate spot area. Overall, 234 *Colias nastes* and 72 *C. hecla* were caught and measured. All measurements were made by CFAB using inverted electronic calipers so that the reading was not seen while the measurement was being taken. Between measurements the calipers were returned to zero.

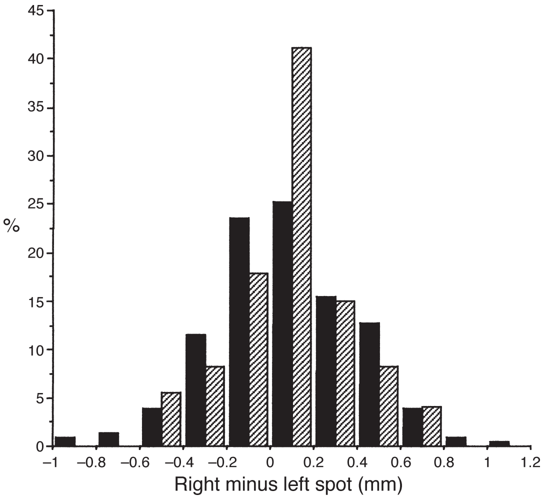

Fig. 1. Histogram showing the distribution of right minus left spot size for *C. nastes* (solid bars) and *C. hecla* (striped bars).

Repeatability measurements were carried out on 16 randomly chosen individuals from each species (eight males and eight females). The right spot was measured four times (taking the same precautions as above between measurements) and repeatability estimates calculated using the method outlined by Lessels and Boag (1987). A mixed-model ANOVA was also used for estimating the repeatability of the asymmetry as a number of authors have suggested that this method may be more appropriate (Palmer & Strobeck 1986, Swaddle *et al.* 1994). Where factors are individuals (I), side (S, right or left) and replicate (R, the repeated measurement) the ratio of the I-by-S mean square to the combined I-by-S-by-R and I-by-R mean squares provides an *F*-test of whether between-individual variation in estimated asymmetry is significantly greater than can be accounted for by measurement error (Swaddle *et al.* 1994). For this, both spots were measured four times for each of the 16 individuals from the two species.

In order to determine whether a trait exhibits fluctuating asymmetry (FA) the frequency distribution of right minus left values should not differ from a normal distribution with a mean of zero (Palmer & Strobeck 1986). To test for this, one sample *t* tests were calculated on absolute asymmetry (right minus left) as well as tests for skew and kurtosis.

Table 1. Descriptive statistics for spot distributions in each species.

	C. nastes	*C. hecla*
Mean (mm)	0.0771 (± 0.0218)	0.076 (± 0.0312)
Variance	0.111	0.071
Kurtosis	0.571	0.093
Skew	–0.040	0.114
t test	$t = 3.54$ $P < 0.001$	$t = 2.43$ $P < 0.05$

Wilcoxon signed rank tests were carried out to determine direction of asymmetry.

To examine the relationship between trait symmetry and size, correlation coefficients were calculated for absolute asymmetry and mean spot size ([right value + left value]/2). We used the *F* test for testing for differences in the variance of FA between samples (Palmer & Strobeck 1992).

All statistics were performed using the programme StatviewSE + Graphics (version 1.02, Abacus Concepts, Inc.).

3. Results

An unpaired two-tailed *t*-test between the absolute asymmetry of males and females was not significant for either species (*C. nastes*: $t_{1,232} = 1.12$, $P = 0.26$; *C. hecla*: $t_{1,71} = 1.34$, $P = 0.18$). Therefore measurements for males and females were pooled in all further calculations.

Repeatability estimates (r) of spot size were high for both species, where possible values of r range from zero to one indicating unrepeatability and perfect repeatability respectively (*C. nastes*: $r = 0.90$, $F_{15,48} = 38.05$ $P < 0.001$; *C. hecla*: $r = 0.94$, $F_{15,48} = 63.15$, $P < 0.001$). A mixed-model ANOVA revealed that between-individual variation in estimated asymmetry was significantly greater than could be accounted for by measurement error (*C. nastes*: $F_{15,90} = 3.04$, $P < 0.001$; *C. hecla*: $F_{15,90} = 4.42$, $P < 0.001$).

The sample mean of right minus left spot values was significantly different from zero (Fig. 1 and Table 1). A Wilcoxon signed rank test showed that this was due to directional asymmetry (*C. nastes*: $z = -3.45$, $P = 0.0006$; *C. hecla*: $z = -2.38$, $P = 0.017$). Thus, both species show weak directional asymmetry for the character investigated.

There was no significant correlation between mean spot size and absolute asymmetry in *C. nastes* ($R^2 = 0.01$), but in *C. hecla* large spots tended to be more symmetrical than small spots ($R^2 = 0.07$, $P < 0.05$).

4. Discussion

We have shown that in two closely related (Brunton 1998) species of *Colias* butterflies a bilateral trait (wing spots) shows directional asymmetry instead of fluctuating asymmetry. Moreover, this result cannot be accounted for in terms of measurement error. Our finding adds weight to the caution provided by Kraak (1997), namely that stringent tests are necessary to demonstrate that asymmetries really are fluctuating.

Given that our two species are closely related (Brunton 1998) we can only suppose one evolution of directional asymmetry has occurred. It remains to be seen whether this pattern is repeatable within the group and within the Lepidoptera more generally. The functional significance of the directional asymmetry is unknown.

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References

Brunton, C. F. A. 1998: The evolution of ultraviolet patterns in European *Colias* butterflies (Lepidoptera, Pieridae): a phylogeny using mitochondrial DNA. — *Heredity* 80: 611–616.

Evans, M. R., Martins, T. L. F. & Haley, M. P. 1995: Inter- and intra-sexual patterns of fluctuating asymmetry in the red-billed streamertail: should symmetry always increase with ornament size? — *Behav. Ecol. Sociobiol.* 37: 15–23.

Kraak, S. B. M. 1997: Flucutuating around directional asymmetry? — *Trends Ecol. Evol.* 12: 230.

Lessels, C. M. & Boag, P. T. 1987: Unrepeatable repeatabilities: a common mistake. — *The Auk* 104: 116–121.

Moller, A. P. 1990: Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. — *Anim. Behav.* 40: 1185–1187.

Moller, A. P. & J. P. Swaddle 1997: *Asymmetry, developmental stability and evolution.* — Oxford University Press, Oxford.

Palmer, A. R. & Strobeck, C. 1986: Fluctuating asymmetry: measurement, analysis, patterns. — *Ann. Rev. Ecol. Syst.* 17: 391–421.

Palmer, A. R. & Strobeck, C. 1992: Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. — *Acta Zool. Fennica* 191: 57–72.

Swaddle, J. P., Witter, M. S. & Cuthill, I. C. 1994: The analysis of fluctuating asymmetry. — *Anim. Behav.* 48: 986–989.