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The effect of conservation efforts on morphological asymmetry in a butterfly population

Dirk S. Schmeller^{a,*}, Matthias Dolek^b, Adi Geyer^b, Josef Settele^c, Roland Brandl^d

^a Station d'Ecologie Expérimentale du CNRS à Moulis, 09200 St. Giron, France

^b Ökologische Forschung und Planung, Obere Dorfstr. 16, 82237 Wörthsee, Germany

^c UFZ – Helmholtz Centre for Environmental Research, Department of Community Ecology, Theodor-Lieser-Str. 4, 06120 Halle/Saale, Germany

^d Philipps-University Marburg, Dept. of Biology, Fac. of Ecology, Karl-v.-Frisch-Str., 35043 Marburg, Germany

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ABSTRACT

There are many biological factors that influence the developmental stability and therewith the morphological symmetry of species, such as the environment, stress during development, hybridisation between species, inbreeding and loss of genetic variability. Here, we analysed the developmental stability of wing traits of the butterfly *Parnassius apollo*, a threatened species with small local populations. We assessed the historical trajectory of developmental stability as measured by fluctuating asymmetry (FA) to evaluate the effect of protection and management actions on an Apollo population in Germany. We analysed 89 individuals collected from 1906 to 2004 at six morphological wing traits, four of which were FA traits. Our results show that legal protection (= listed on a red list) alone did not have any effect on FA and hence did not improve the population fitness. However, FA showed a clear response to management actions, but only after several generations. In 2004, 13 years after population management actions were implemented, the variance of population wide FA was comparable to the FA-variance from the beginning of the 20th century. Our study supports the utilisation of FA as an assessment tool of effects of population management.

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Introduction

Demography and genetic variability are among the important determinants for long-term survival of populations (Caughley & Gunn 1996; Hoffmann & Woods 2001; Soulé 1987). Habitat fragmentation leads to small populations, which are therefore not only threatened by the vagrancies of demographic stochasticity but also by the loss of genetic variability due to genetic drift (Lande & Barrowclough 1987; Lande & Shannon 1996). This loss of genetic variability may lead to a decrease of fitness (Reed & Frankham 2003; Saccheri et al. 1998). However, it is time-consuming and sometimes impossible to estimate individual or population fitness. Therefore, evolutionary biologists suggested a number of surrogate as morphometric surrogates of fitness, such as fluctuating asymmetry (FA) of bilateral traits (Anciaes & Marini 2001; Leamy 1999; Lens & Van Dongen 2000; Lens et al. 2002). The development of both sides of a bilateral trait is considered to

be based on the same set of genes (e.g. Clarke 1993) and can result in several forms of asymmetries, including antisymmetry, directional asymmetry and fluctuating asymmetry. While antisymmetry and directional asymmetry supposedly are inherited (i.e. Palmer & Strobeck 1992), random differences between the two sides (FA) represent phenotypic variation, resulting from genotype \times environment interactions. Fitness as determined by FA is then a measure of developmental stability indicating the ability of an individual to buffer against stress during its development (Palmer & Strobeck 1992).

There are many biological factors that influence asymmetry of bilateral organisms, such as the environment, stress during development, hybridisation between species, inbreeding and loss of genetic variability (Bechshøft et al. 2008; Palmer & Strobeck 1992). Especially at the edge of the distributional range of a species these factors work together (Kark et al. 2004). For *Turdus helleri*, Lens et al. (2002) have clearly shown that the degree of asymmetry of individuals correlated with the survival of a species. Many other studies examined the effect of environmental conditions, area of suitable habitats (Anciaes & Marini 2001; Teixeira et al. 2006), pollution (Bonada et al. 2005; Bonada & Williams 2002; Ostbye et al. 1997) or contamination of the environment (Oleksyk et al. 2004; Sonne et al. 2005) and more generally habitat quality (Weller & Ganzhorn 2004) on FA of morphological traits.

* Corresponding author.

E-mail addresses: ds@die-schmellers.de (D.S. Schmeller),

Matthias.Dolek@Geyer-und-Dolek.de (M. Dolek), Adi.Geyer@Geyer-und-Dolek.de (A. Geyer), josef.settele@ufz.de (J. Settele), rbrandl@staff.uni-marburg.de (R. Brandl).

So far, no study estimated FA before and after the implementation of management strategies in a population, despite the suggestion that FA may be a tool to monitor the effect of the implementation of management plans (i.e. Ostbye et al. 1997). The quantification of such impacts and the population's reaction time to management plans would provide valuable information to evaluate current and future management plans. This is especially true for rare and threatened species, such as the Apollo butterfly *Parnassius apollo*. Agricultural changes, led to (i) a reduction of open habitats, (ii) a decrease in grassland and rock habitats, and (iii) an increase in forest cover. These factors, which all increase fragmentation and isolation of populations, partly account for the decline of the Apollo butterfly in Europe (Witkowski et al. 1997). In Germany, the loss of the caterpillar's host plant caused by overgrowth of more competitive herbs and mosses is among the most important reasons for the drastic decline of *P. apollo* and its patchy distribution (Binot et al. 1998; Chrostowski 1958; Descimon 1995; Gauckler 1963; Geyer & Dolek 2001). The decline is likely linked to reduced dispersal and gene flow between subpopulations. Several studies have shown that habitats of low quality hamper dispersal of *Parnassius* species and that they need open grasslands for dispersal, avoiding forest edges (Matter et al. 2009; Matter et al. 2004). At the beginning of the 20th century the *P. apollo* had a much broader distribution in Germany and adjacent countries, while currently its occurrence is patchy despite some management programs (Adamski & Witkowski 2002). In Germany, the Apollo occurs in only a few habitats in the Alps, in the Jura Mountains in southern Germany, and along the Mosel river (Dolek & Geyer 2000; Dolek & Geyer 2002; Geyer & Dolek 1999; Geyer & Dolek 2001). The Apollo butterfly was red-listed as vulnerable (VU) in 1996 (Gimenez Dixon 1996) and now is categorised as near threatened (NT) on the IUCN Red List (Van Swaay et al. 2010).

Here, we assess the historical trajectory of developmental stability as measured by FA to evaluate the effect of protection and management actions on an Apollo population in Germany. In contrast to a study on a Polish population of *P. apollo*, showing an increase of FA after an assumed genetic bottleneck in the 1960s (Adamski & Witkowski 2002), we can make a direct link with habitat improvements in a specific population in the German Franconian Alps. That population was legally protected in 1936 (Pretscher 1998) and in 1990 habitat management activities were started (Dolek & Geyer 2002). The availability of museum samples from the beginning of the 20th century and recent samples from the same population represents an excellent opportunity to characterise the changes in FA over almost one century.

Materials and methods

The Apollo butterfly is a stenophagous butterfly found in mountainous regions and is restricted to habitats on nutrient-poor calcareous grasslands, rocky vegetation, and rocky slopes with nectar rich food plants, such as thistles and knapweeds (Binot et al. 1998). In Germany, caterpillars are monophagous and feed on the white stonecrop *Sedum album*, while in other parts of Europe they feed also on the purple (*S. telephium*; Nakonieczny & Kedziorski 2005) or the blue spruce stonecrop (*S. reflexum*; Brommer et al. 1998). In 1990, management activities started in the focal population, including grazing by sheep and goats as well as rock exemption and tree culling (Dolek & Geyer 2002). The population experienced an increase in larval numbers per *Sedum* plant until the year 1998, where a cold period in June led to larval death. The population only recovered slowly and reached a maximum larval density in 2003 (Fig. 1).

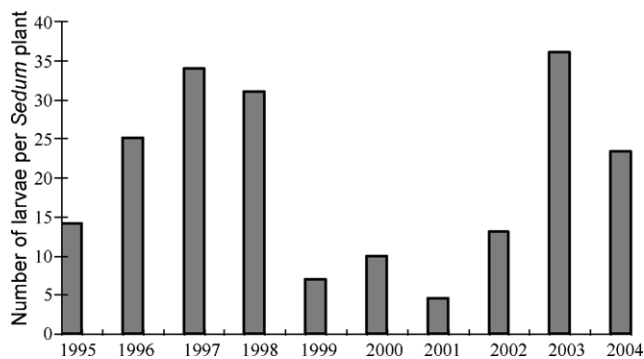


Fig. 1. Larval density of *Parnassius apollo* on *Sedum album* fodder plants in the highest quality habitat patch of the population.

Morphological measurements and measurement error

We measured six bilateral wing traits (Fig. 2) of 89 sexed individuals of *P. apollo* (34 females, 55 males) from populations in the Northern Franconian Jura (Bavaria, Germany). Individuals from 1906 to 1968 stem from the collection of the zoological



Fig. 2. Illustration of the measured wing traits. 1 (t1) = diameter of red central wing spot along vein m₂, 2 (t2) = diameter of black coloration of central wing spot along vein m₂, 3 (t3) = length of vein of the discoidal cell from junction of vein m₁ to junction of vein m₂, 4 (t4) = length of vein of the discoidal cell from junction of vein m₁ to junction of vein rr, 5 (t5) = length of vein cu₂ from discoidal cell to the edge of the wing, 6 = forewing: distance between branching of veins m₃ and r₃. All measurements had been taken as straight lines between measurement points.

Table 1

Error analysis following (Palmer 1994) using a two-way, mixed ANOVA model (sides \times individuals). We report the mean square of the measurement error (MS_{ME}), the F -value (F), the Kolmogoroff–Smirnov's d -value ($KS\ d$), and the level of significance (p) of each test. FA-traits are marked bold face.

	<i>df</i>	Type III SS	MS	<i>F</i>	<i>p</i>
t1					
Side	1	0.099	0.099	3.6	0.066
ID	16	126.839	7.927	286.74	<0.0001
Side \times ID	16	4.041	0.253	9.13	<0.0001
MS (Error)	34	0.940	0.028		
Mean	8.803				
<i>KS d</i>	0.165, $p > 0.15$				
t2					
Side	1	0.107	0.107	2.71	0.109
ID	16	141.794	8.862	224.02	<0.0001
Side \times ID	16	4.140	0.259	6.54	<0.0001
MS (Error)	34	1.345	0.040		
Mean	11.143				
<i>KS d</i>	0.153, $p > 0.15$				
t3					
Side	1	0.038	0.038	6.74	0.014
ID	16	10.218	0.639	114.28	<0.0001
Side \times ID	16	3.532	0.221	39.51	<0.0001
MS (Error)	34	0.190	0.006		
Mean	3.694				
<i>KS d</i>	0.279, $p < 0.01$				
t4					
Side	1	0.053	0.053	2.56	0.119
ID	16	57.745	3.609	174.05	<0.0001
Side \times ID	16	3.589	0.224	10.82	<0.0001
MS (Error)	34	0.705	0.021		
Mean	6.484				
<i>KS d</i>	0.127, $p > 0.15$				
t5					
Side	1	0.002	0.002	0.05	0.822
ID	16	112.696	7.043	153.51	<0.0001
Side \times ID	16	3.393	0.212	4.62	<0.0001
MS (Error)	34	1.560	0.046		
Mean	15.312				
<i>KS d</i>	0.167, $p > 0.15$				
t6					
Side	1	0.025	0.025	0.06	0.808
ID	16	261.939	16.371	39.52	<0.0001
Side \times ID	16	3.468	0.217	0.52	0.916
MS (Error)	34	14.085	0.414		
Mean	8.428				
<i>KS d</i>	0.18, $p > 0.05$				

state collection in Munich. The samples from 1995 to 2004 were collected in the field. Dried specimen (sampled between 1906 and 1968) were measured in the laboratory. The samples from recent years (1995–2004) were measured in the field using the same stereomicroscope as in the laboratory and were released afterwards.

We followed the suggestions of Palmer (1994) and Palmer and Strobeck (2003) to estimate the measurement error and asymmetries, using a mixed model, two-way ANOVA (SAS Institute Inc., proc glm SAS 9.1.3). This procedure allows testing for directional asymmetry (as the effect of side) and measurement error (error MS) simultaneously (Palmer 1994). We determined the measurement error on a dataset of 17 individuals measured twice at each trait. The drying process of museum samples should not have altered FA, as water content in butterfly wings is usually very small (Talloon et al. 2004). However, to rule out any confounding factors of the drying process in museum samples, we did double measures on individuals across the whole sampling period, including dried and live animals. We further only used well preserved individuals to avoid difficulties in finding start and end points of traits. In a second step, we used Kolmogoroff–Smirnov tests for normal distribution to rule out antisymmetry (Palmer & Strobeck 2003). We finally employed Pearson correlations to detect intercorrelations between traits.

Statistical analysis

As legal protection and management changed over the years, we grouped our samples into five decades; 1910 ($N=6$), 1930 (14), 1970 (19), 2000 (41), 2010 (9). As FA in our sample of butterfly individuals may be the result of gender effects, and/or protection or management status, we explored the potential effects on untransformed measurements for each trait using ANOVAs, with side and decade as fixed and gender as random effect, but did not find support for a gender effect (see also Adamski & Witkowski 2002). For the remaining analyses, we therefore pooled all individuals. Differences in the means of FA between decades were tested with a post-hoc analysis based on Tukey's HSD test (SAS Institute Inc., proc glm SAS 9.1.3).

Results

A total of 89 individuals were analysed at six traits. The results of the two-way mixed ANOVAs on repeated measurements (side \times individual) confirmed that in all, but trait 6, between-side variation was larger than the measurement error. Kolmogoroff–Smirnov tests on signed differences of R-L values showed that all, except trait 3, were normally distributed (Table 1). Hence, traits 1, 2, 4, and 5 represent FA traits. Signed R-L values

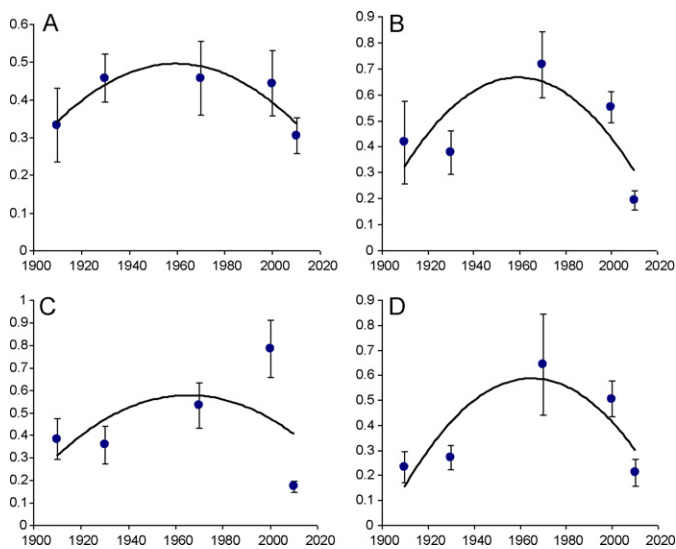


Fig. 3. Historical trajectory of FA in *Parnassius apollo* in the four FA traits (t1 = A, t2 = B, t3 = C, t4 = D). Each mean value by decade is shown with its standard error.

Table 2
Results of Tukey's HSD post-hoc test for significant mean differences of FA between the analysed decades. Comparisons significant at the 0.05 level are indicated by x. Trait a is in the lower diagonal, trait b in the upper.

	1910	1930	1970	2000	2010
Trait 1/2					
1910	–	ns	ns	ns	x
1930	ns	–	ns	ns	x
1970	ns	ns	–	–	x
2000	ns	ns	x	–	x
2010	x	x	x	x	–
Trait 4/5					
1910	–	x	x	x	x
1930	ns	–	ns	ns	ns
1970	ns	ns	–	ns	ns
2000	ns	ns	ns	–	ns
2010	ns	x	x	x	–

of traits 2 and 5 showed a weak correlation (Pearson $p = 0.0453$), while the remaining pairs of traits were uncorrelated ($p > 0.289$).

Over decades, the different traits showed slightly different trajectories (Fig. 3), but all showed comparable or lower levels of FA in the samples of the decade 2010 as compared to 1910. In trait 1, FA increased after 1910 and stayed relatively stable from decades 1930 to 2000 and dropped to the same level than in 1910 in the last decade 2010. Traits 2 and 5 both showed a maximum FA and strong variance in 1970. While in trait 5 FA in 2010 was comparable to 1910, FA dropped even below that level in trait 2. Finally, trait 4 showed a maximum FA in 2000, with a strong decrease thereafter below the level of 1910. Differences between decades were found to be significant in all traits ($F_5 = 7.64, p < 0.001$). The post-hoc tests showed that significant mean differences in FA occurred mainly between 1910, respectively 2010, and the other decades (Table 2).

Discussion

We analysed asymmetry of 89 *P. apollo* specimens from 1906 to 2004 using six bilateral wing traits, of which four matched the criteria of fluctuating asymmetry (FA) and one of directional asymmetry (DA; Palmer & Strobeck 2003). Our results suggest that legal protection alone may have little effect on population fitness, and that management actions are imperative, especially for highly isolated and small, remnant populations as frequently found in the

Apollo butterfly and closely related species (Bengtsson et al. 1989; Roland et al. 2000; Witkowski & Adamski 1996).

In 1936, the Apollo butterfly was legally protected in Germany to conserve the remaining populations. However, our data showed that the FA of the investigated population did not change with the protection status. On the contrary, we found a significantly higher variance of FA in the 1970s and 2000s, similar to findings of a small and isolated Polish population (Adamski & Witkowski 2002). Our results indicate that population viability did not improve despite the legal protection. Our hypothesis is based on the fact that a smaller population size is associated with increased stress and loss of genetic variability, which altogether may increase FA (e.g. Bechshøft et al. 2008; Britten 1996; Clarke & Oldroyd 1996; Kristensen et al. 2003). Despite the protection in 1936, grazing regimes were not optimised for the conservation of the Apollo populations, allowing forestation of open grasslands and overgrowth of rocks by mosses, herbs, and shrubs. Dispersal of a sister species of *P. apollo*, *P. smintheus*, was severely limited by such conditions (Matter et al. 2009). Hence, only in combination with appropriate management strategies with optimised grazing regimes of sheep and goats the populations of thistles, knapweeds, and suitable *S. album* increased within the habitats of the Apollo and kept open grasslands, necessary for the dispersal of *P. apollo* (see also Brommer & Fred 1999; Matter & Roland 2002).

It also needs to be kept in mind that despite improvements due to habitat management, the population remained vulnerable to disturbance, as the population development shows (Fig. 1). The larval number declined heavily after a cold spell during the larval period in 1998, from which it took several years to recover. That vulnerability, likely the result of the severe bottleneck in the 1960s, may also account for the slow response to the management strategies in general. Only 13 generations after management actions were established a significant decrease of FA across the individuals of a population was evident. It is very likely that sister species of *P. apollo*, inhabiting a similar ecological niche and showing a similar population structure (i.e. *P. smintheus*; Matter & Roland 2002; Roland et al. 2000), are prone to the same pressures and same fitness reductions as shown here. It is likely that reduced dispersal and habitat connectivity due to, i.e. forest barriers (Roland et al. 2000) will lead to an isolation of populations with subsequent reduction in size, depletion of genetic variability and loss of fitness. Hence, we agree with earlier suggestions (Roland et al. 2000; Witkowski et al. 1997) that conservation efforts should be directed toward the promotion of habitat connectivity, as we were able to prove here that these kind of management strategies increase the population fitness of *P. apollo*.

In conclusion, we showed that legal protection alone had little effect on FA whereas a combination of protection and management decreased the variance of FA. If FA is a surrogate of fitness, this finding may explain why populations often continue to decline, despite legal protection (e.g. *Lutra lutra*; Jefferies 1989). Conservation of species must, hence, include sound strategies of population management (such as increase of habitat connectivity and quality), especially in species with small and remnant populations. Furthermore, our study implies that FA measures could be used to monitor populations (Leary & Allendorf 1989; Lens et al. 2002), complementing existing monitoring programs on population dynamics. The additional assessment of FA in smaller and more isolated populations could be a useful tool to indicate a breaking point of a population, when more rigorous conservation measures are required for the survival of that population. To set such a breaking point museum collections provide valuable information on the historical trajectory of populations. Comparisons of recent samples with historical specimen may then give additional information on the temporal changes in the fitness of populations. We also suggest more mechanistic and experimental approaches to advance

the knowledge of fluctuating asymmetry beyond its current state to make it a useful conservation tool.

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