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Developmental instability in sexually reproducing and parthenogenetic populations of *Bacillus rossius rossius* and *Bacillus rossius redtenbacheri*

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ABSTRACT

Developmental instability, estimated by fluctuating asymmetry and morphological variance, was investigated in sexually reproducing and parthenogenetic populations of *Bacillus rossius rossius* and *Bacillus rossius redtenbacheri*. Fluctuating asymmetry was significantly higher in parthenogenetic females than amphigonic females in both sub-species. It was also higher in males and parthenogenetic females of the sub-species *redtenbacheri* than in males and parthenogenetic females of the sub-species *rossius*. When we compared fluctuating asymmetry between males and females in amphigonic populations within each sub-species, we found no significant differences with one exception: the antenna showed higher fluctuating asymmetry in males than in females in both sub-species. There was a higher morphological variance in parthenogenetic females of the sub-species *rossius* than in parthenogenetic females of the sub-species *redtenbacheri*, whereas no significant differences were found between the amphigonic females of the two sub-species. In both sub-species, there was evidence for higher morphological variance in amphigonic than parthenogenetic females. When Lerner's conjecture was tested, fluctuating asymmetry was not significantly higher in individuals with a body size more than one standard deviation from the mean of the size distribution.

Keywords: amphigonic reproduction, developmental instability, fluctuating asymmetry, parthenogenetic reproduction.

INTRODUCTION

Sexually reproducing individuals produce genetically diverse offspring that may be less prone to extinction than genetically uniform offspring in variable environments (see review in Williams, 1975). Hence, sexual reproduction maintains genetic flexibility in a population, especially in variable environments, where different combinations of genes may respond differently to environmental fluctuations. On the other hand, parthenogenesis, which has

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evolved independently in many animal and plant groups (White, 1973), has the advantage that clonally reproducing individuals maximize their genetic representation in future generations. However, in parthenogens, adaptive gene combinations are maintained at the expense of producing genetically uniform offspring. Under optimal conditions, parthenogenetic individuals have twice the fitness advantage of sexually reproducing individuals, but the cost of clonal reproduction is high in a variable environment (Maynard Smith, 1978; Hurst and Peck, 1996; Barton and Charlesworth, 1998). To quantify the relative cost and consequences of clonal reproduction in a variable environment, we compared developmental instability in sexually reproducing and parthenogenetic populations of two Italian sub-species of *Bacillus rossius*.

Developmental instability, which is influenced by several factors (e.g. pollution, stress during development, genetic stress), appears to be more or less correlated with a number of fitness-related traits (see Møller and Swaddle, 1997, and references therein; but see Clarke, 1997a). Furthermore, it has been suggested that developmental instability is greater in sexually selected traits than in other traits (Møller and Thornhill, 1998; but see Palmer 1999). The genetic basis of developmental instability and canalization is generally unknown, even though many studies have focused on this topic (for canalization, see Wagner *et al.*, 1997; for developmental instability, see Clarke, 1993). No convincing genetic mechanism for developmental instability has been suggested, but hypotheses regarding the genetic and molecular mechanisms of canalization have been proposed. Thoday (1958) proposed that modifier genes could be responsible for the maintenance of canalization of a trait; recent research of heat-shock protein HSP90 mutants in *Drosophila* has provided the first molecular evidence for such a mechanism (Rutherford and Lindquist, 1998).

Bacillus rossius is a very peculiar stick insect, which, over its wide holomediterranean range, builds up both bisexual and facultatively parthenogenetic, all-female populations. The latter obviously derive from nearby amphigonic demes, since their genetic structure reflects more closely that of their nearest bisexuals rather than that of geographically distant parthenogens; two instances of actual shift from bisexual to unisexual reproduction have been witnessed (Tinti, 1993; Scali, 1996). The two sub-species are *B. rossius rossius*, which is spread along the western coast of Italy and throughout Corsica and Sardinia, and *B. rossius redtenbacheri*, which ranges from Sicily and the Adriatic/Ionian coasts of Italy to Croatia, Yugoslavia, Albania and Eastern Greece. About 30 populations, belonging to both sub-species, were analysed for 20 gene–enzyme systems: *Ald*, *aGpdh* (monomorphic); *Adk-1*, *Adk-2*, *Fh*, *Got-1*, *Got-2*, *Gox*, *G6pdh*, *G3pdh*, *Hk-1*, *Hk-2*, *Idh-1*, *Idh-2*, *Mdh-1*, *Mdh-2*, *Mpi*, *6Pgdh*, *Pgi*, *Pgm* (polymorphic) (Nascetti and Bullini, 1983; Scali and Mantovani, 1989; Mantovani and Scali, 1991; Tinti *et al.*, 1992). Heterozygosity estimates from samples that also included populations close to the four under study (see below) gave values of 0.062 for *B. rossius rossius* and 0.033 for *B. rossius redtenbacheri*. Only bisexual samples contribute to heterozygosity, since heterozygous unisexuals are rare due to the parthenogenetic reproduction mechanism that doubles the A + X set of the reduced egg through anaphase restitution to produce a thelytokous offspring homozygous at all loci (Pijnacker, 1969; Scali, 1969).

Two principal methods are commonly used for the estimation of developmental instability: morpholoigcal variance (Zouros *et al.*, 1980; King, 1984; Livshits and Kobyliansky, 1984; Imasheva *et al.*, 1997), even if the estimate can be blurred by additive genetic variation, and fluctuating asymmetry (see Møller and Swaddle, 1997, and references therein).

Some studies have found a negative correlation between heterozygosity and developmental instability using both methods; however, others have failed to find such a relationship (for reviews, see Mitton, 1995; Britten, 1996). Analysis of differences in fluctuating asymmetry between males and females of haplo-diploid taxa has been limited to a few species only (Clarke *et al.*, 1986, 1992; Clarke, 1997b; Crespi and Brett, 1997) and no clear patterns have emerged.

The main aim of the present study was to determine whether there is a genetic relationship between genetic diversity (heterozygosity) and developmental instability (fluctuating asymmetry or morphological variance). We tested this hypothesis in three ways: first, by comparing the amphigonic males and females of two sub-species with different levels of heterozygosity; second, by comparing parthenogenetic and amphigonic females within the same sub-species, with the parthenogenetic individuals being totally homozygous; third, by comparing the fluctuating asymmetry of individuals at the extremes of the size distribution with that of the modal phenotypes. Such a comparison is based on the suggestion that homozygous individuals are more likely to belong to classes with extreme phenotypes and thus should have greater developmental instability (Lerner, 1954). We also wished to establish whether there are differences in developmental instability (fluctuating asymmetry and morphological variance) between the sexes within the same sub-species.

MATERIALS AND METHODS

Collection of specimens

Stick insects are best collected at night, when adults actively feed, mate and lay eggs. During October 1997, we collected 162 *B. rossius rossius* specimens along the Tyrrhenian coast: 68 parthenogenetic females and 94 amphigonic specimens (41 females, 53 males). The parthenogenetic population was collected at Grilli (40 km south of Grosseto, Tuscany) in a degraded zone where the population lives on bramble bushes. The amphigonic sample was collected at Capalbio, about 15 km south of Grilli, where Mediterranean vegetation is much better preserved and the stick insects mainly live and feed on lentisk shrubs, but also on ilex, myrtle and heather. During the same month, we collected 119 *B. rossius redtenbacheri* specimens along the Adriatic coast, at about the same latitude as that of the Tuscan populations: 68 parthenogenetic females, plus 21 females and 30 males of an amphigonic population. The parthenogenetic sample was collected at Villa Rosa (50 km north of Pescara, Abruzzo) in a degraded zone where the population also lives on bramble along a busy road. The amphigonic sample was collected at Torino di Sangro Marina (40 km south of Pescara) in a pine wood area where the insects feed on lentisk, hornbeam, oak and bramble shrubs.

Measurements and statistical analysis

We measured five bilateral traits and three unilateral traits. The bilateral traits were measured using a binocular microscope with a digital filar eyepiece (Los Angeles Scientific Instrument Company, Inc., USA). The following traits were considered: the antenna, the labial palpus, the maxillary palpus, the length of the fore-legs without the segmented tarsus (femur and tibia) and the cercus on females. Asymmetry was estimated as the difference in

length between each bilateral pair of traits (right – left side). Fluctuating asymmetry was calculated as the variance of (right – left), as an absolute value and as a mean value (Palmer and Strobeck, 1986; Palmer, 1994). A two-way analysis of variance was conducted to test for the significance of fluctuating asymmetry relative to measurement error (following Palmer, 1994). Some individuals showed a strong asymmetry exceeding three standard deviations of the signed values of (right – left) on antenna because of a reduction in segments. These individuals were considered phenodeviants and the fluctuating asymmetry values of their antenna were not included in the analysis.

The unilateral traits (mesonotum, metanotum and abdomen) were measured with a digital calliper to the nearest 0.1 mm. In an effort to quantify possible errors when measuring these traits, we chose 20 amphigonic individuals (10 males and 10 females) at random and for each individual we measured the three traits 10 times. The within-individual coefficient of variation for each mean was then taken as an estimate of the measurement error, adding Haldane's (1955) correction for small sample size. The measurement error of the three traits was low with a mean value of 0.13%.

In all analyses, sub-species, populations, sexes and amphigonic and parthenogenetic females were separated and analysed independently. Variances of the unilateral traits and of the difference in length between each bilateral pair as right minus left (right – left) were calculated. An *F*-test (Fowler and Cohen, 1990) was conducted to compare the variances. We did not perform any log-transformation as the traits were normally distributed and we compared variances instead of coefficients of variation (CV), as the range of the traits' size was very narrow.

To check if the data obtained displayed the statistical properties of fluctuating asymmetry (an approximately normal distribution of signed asymmetry scores around a mean of zero), the hypothesis that the mean of right minus left character values equals zero was tested using a one-sample *t*-test. Normality was tested using Lilliefors' test and by inspecting the distributions graphically (following Palmer, 1994).

A Spearman rank correlation (Zar, 1984) was used to test whether the absolute fluctuating asymmetry of the bilateral traits was correlated with the sum of the mesonotum, metanotum and abdomen. The sum was taken as an estimate of the body size of the insect (following Palmer, 1994). A Spearman rank correlation was used to test whether the fluctuating asymmetries of different traits were correlated.

A sequential Bonferroni test (Rice, 1989) was applied to avoid significant results arising as a consequence of a large number of related tests. Following Miller's (1981) suggestions, we made a separate probability statement for each sub-species (when the reproductive systems were tested) or for each reproductive system (when the two sub-species were compared).

Lerner's conjecture

To test the prediction that the most extreme phenotypes of each group should also show the greatest fluctuating asymmetry (Lerner's conjecture), mean values of the insects' body size were calculated in each group. The fluctuating asymmetry of individuals with trait values within one standard deviation of the mean was compared with that of those with trait values outside the range using a *t*-test and a non-parametric Mann-Whitney *U*-test (Zar, 1984; Hoffmann *et al.*, 1999).

RESULTS

Measurement error

The interaction 'mean square' (MS), which contains information about fluctuating asymmetry, was tested against error mean square (reflecting measurement error). Fluctuating asymmetry MS was significantly larger than measurement error MS in all cases ($2.3 <$ interaction MS < 6.1; $0.09 <$ error MS < 0.14; d.f. $= 164$, $P < 0.001$) bar one, the cercus (interaction MS $= 0.35$, error MS $= 0.38$; d.f. $= 164$, $P > 0.05$), which was excluded from further analysis.

Fluctuating asymmetry

No significant deviations from zero of the mean of the trait (right – left) distributions were found for the antenna (one-sample *t*-test: $0.11 < P < 0.68$; $19 <$ d.f. < 65). The labial palpus showed two significant deviations from zero (one-sample *t*-test: $0.008 < P < 0.8$; $20 <$ d.f. < 67), the maxillary palpus one significant deviation from zero (one-sample *t*-test: $0.01 < P < 0.1$; $20 <$ d.f. < 67) and the fore-leg one significant deviation from zero (one-sample *t*-test: $0.02 < P < 0.7$; $20 <$ d.f. < 67). We decided, however, not to exclude the traits in which fluctuating asymmetry showed a significant deviation from zero, because the deviations could have been due to the small sample sizes and the significance vanished if we removed one or two extreme values.

Deviations from normal distributions were not significant at the 0.01 level for almost all of the traits (Lilliefors' test: $0.02 < P < 0.58$; $20 < n < 68$). Only 6 of 24 Lilliefors' tests gave significant deviations from normality at the 0.05 level. Hence, the distributions of all traits' fluctuating asymmetry were inspected graphically; the deviations from normality were due to a leptokurtic distribution. Only the fore-leg showed a platykurtic distribution with peaks at the extreme values, thus exhibiting anti-symmetry. Therefore, we removed the fore-legs from our analysis.

The fluctuating asymmetries of the different traits did not appear to be correlated ($-0.36 < r_s < 0.32$; $20 < n < 68$; $0.08 < P < 0.95$). No correlations were found between the sum of the mesonotum, metanotum and abdomen (body size of the insect) and the fluctuating asymmetry of the traits ($-0.44 < r_s < 0.35$; $16 < n < 55$; $0.06 < P < 0.97$).

Fluctuating asymmetry between the two sub-species

Some significant differences in variance (*F*-test) of the signed values of (right – left) of the bilateral traits were noted between the different sub-species (see Table 1). In particular, we found evidence for greater fluctuating asymmetry in the males of the *redtenbacheri* sub-species than the males of the *rossius* sub-species. For amphigonic females only, the fluctuating asymmetry of the maxillary palpus was greater in the *redtenbacheri* than the *rossius* sub-species. Parthenogenetic females had greater fluctuating asymmetry in the sub-species *redtenbacheri* than in *rossius* (Table 1); all significant results shown in Table 1 were still significant after a sequential Bonferroni test ($P < 0.05$, $K = 3$).

Fluctuating asymmetry between the two reproductive systems within sub-species

Significant differences in variance (*F*-test) of the signed values (right – left) of the bilateral traits were found between the two different reproductive systems (see Table 2). In particular, we found strong evidence for a greater fluctuating asymmetry in the parthenogenetic females than in the amphigonic females of the *rossius* sub-species. The same was found for the *redtenbacheri* sub-species, with the exception of the *labial palpus*, for which the fluctuating asymmetry was greater in the amphigonic than in the parthenogenetic females (Table 2). All significant results shown in Table 2 were still significant after a sequential Bonferroni test ($P < 0.05$, $K = 3$).

Fluctuating asymmetry between the sexes within sub-species

When we compared the fluctuating asymmetry between males and females within sub-species, the only significant difference was for the antenna, which had greater fluctuating asymmetry in males than in females in both sub-species (Table 2).

Morphological variance

Morphological variance between the two sub-species

Significant differences in variance (*F*-test) of the unilateral traits were found between sub-species. The morphological variance of parthenogenetic females was higher in the *rossius* sub-species than in the *redtenbacheri* sub-species; no significant differences were

Table 1. *F*-tests of the variances of the signed values (right – left) of the length of the antenna, labial palpus and maxillary palpus within the two sub-species *Bacillus rossius redtenbacheri* and *Bacillus rossius rossius*

	B. r. redtenbacheri	*B. r. rossius*	*F*-test
Parthenogenetic females			
Antenna	1.23E-4 (66)	6.59E-5 (65)	1.87 (+)**
Labial palpus	1.73E-4 (68)	5.32E-5 (65)	3.24 (+)***
Maxillary palpus	2.81E-4 (68)	9.79E-5 (65)	2.87 (+)**
Amphigonic females			
Antenna	1.91E-5 (20)	2.42E-5 (38)	1.26
Labial palpus	7.01E-5 (21)	7.05E-5 (41)	1.00
Maxillary palpus	8.71E-5 (21)	4.64E-6 (41)	18.77 (+)***
Males			
Antenna	1.77E-4 (24)	5.11E-5 (47)	3.46 (+)**
Labial palpus	8.69E-5 (30)	5.02E-5 (53)	1.73 (+)*
Maxillary palpus	7.23E-5 (30)	1.99E-5 (53)	3.66 (+)**

Note: *F*-tests were performed between individuals with the same reproductive system. The values shown are the variances; figures in parentheses are sample sizes. In the right-hand column, '+' indicates a higher variance of the trait being considered for the sub-species *redtenbacheri* than for the sub-species *rossius*.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. *F*-test of the variances of the signed values (right – left) of the length of the antenna, labial palpus and maxillary palpus within the two sub-species *Bacillus rossius redtenbacheri* and *Bacillus rossius rossius*

	Parthenogenetic *vs* amphigonic females	Amphigonic females *vs* males
B. r. redtenbacheri		
Antenna	6.43 (+)***	9.26 (–)***
Labial palpus	8.25 (–)***	1.23
Maxillary palpus	21.09 (+)***	1.20
B. r. rossius		
Antenna	2.72 (+)***	2.11 (–)**
Labial palpus	5.53 (+)***	1.40
Maxillary palpus	68.75 (+)***	1.22

Note: The *F*-test was performed between individuals of different sex or with different reproductive systems. The values shown are the *F*-values. The '+' indicates a higher variance of the denominator of the comparison, the '–' indicates a higher variance of the numerator of the comparison.
** $P < 0.01$, *** $P < 0.001$.

found between the amphigonic females of the two sub-species (Table 3). The morphological variance of one trait, the metanotum, was significantly greater in the males of the *redtenbacheri* sub-species than in those of the *rossius* sub-species (Table 3). All significant results shown in Table 3 were, with one exception, still significant after a sequential Bonferroni test ($P < 0.05$, $K = 3$).

Morphological variance between reproductive systems within sub-species

Significant differences in variance (*F*-test) of the unilateral traits between the two reproductive systems were found in both sub-species. There was strong evidence for greater morphological variance among amphigonic females than parthenogenetic females in both sub-species (Table 4). All significant results shown in Table 4 were still significant after a sequential Bonferroni test ($P < 0.05$, $K = 3$).

Morphological variance between the sexes within sub-species

The amphigonic females of both sub-species had greater morphological variance than the males (Table 4), except for the metanotum in the *redtenbacheri* sub-species.

Statistical analysis of Lerner's conjecture

There was no significant difference in fluctuating asymmetry between individuals whose body size values were more than one standard deviation around the mean and those whose body size values were within one standard deviation around the mean (*t*-test: $0.025 < t < 0.355$; $5 < n < 27$; $0.075 < P < 0.98$; Mann-Whitney *U*-test: $32.5 < U < 228$; $5 < n < 27$; $0.06 < P < 0.69$).

Table 3. *F*-tests of the variances of the traits mesonotum, metanotum and abdomen between the two sub-species *Bacillus rossius redtenbacheri* and *Bacillus rossius rossius*

	B. r. redtenbacheri	*B. r. rossius*	*F*-test
Parthenogenetic females			
Mesonotum	0.66 (19)	1.29 (58)	1.95
Metanotum	0.68 (19)	1.38 (58)	2.03 (–)*
Abdomen	3.00 (19)	6.77 (58)	2.26 (–)**†
Amphigonic females			
Mesonotum	2.53 (19)	5.10 (22)	2.01
Metanotum	1.36 (19)	2.79 (22)	2.05
Abdomen	17.23 (19)	14.84 (22)	1.16
Males			
Mesonotum	0.89 (19)	1.10 (29)	1.23
Metanotum	11.33 (19)	0.70 (29)	16.18 (+)***
Abdomen	3.97 (19)	4.49 (29)	1.13

Note: *F*-tests were performed between species with the same reproductive system. The values shown are the variances; figures in parentheses are sample sizes. In the right-hand column, '+' indicates a higher variance of the trait being considered for the sub-species *redtenbacheri* than for the sub-species *rossius*; '–' indicates a higher variance of the trait being considered for the sub-species *rossius* than for the sub-species *redtenbacheri*.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, †result no longer significant after Bonferroni correction, which was conducted holding a different probability statement for each reproductive system.

Table 4. *F*-tests of the variances of the traits mesonotum, metanotum and abdomen within the two sub-species *Bacillus rossius redtenbacheri* and *Bacillus rossius rossius*

	Parthenogenetic females *vs* amphigonic females	Amphigonic females *vs* males
B. r. redtenbacheri		
Mesonotum	(–)**†	(+)*†
Metanotum	N.S.	(–)**†
Abdomen	(–)***	(+)**†
B. r. rossius		
Mesonotum	(–)***	(+)***
Metanotum	(–)*†	(+)***
Abdomen	(–)**†	(+)***

Note: *F*-tests were performed between individuals of the same sub-species with different reproductive systems. '+' indicates a higher variance of the denominator and '–' indicates a higher variance of the numerator in the comparison.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, † result no longer significant after Bonferroni correction, which was conducted holding a separate probability statement for each sub-species.

DISCUSSION

Fluctuating asymmetry

There could be two explanations for the deviations from normality of the unsigned values of fluctuating asymmetry due to an excessive leptokurtosis of the distribution. First, when the unit of measurement is large in comparison with the actual asymmetry, there will be a tendency for the population's asymmetry distribution to show leptokurtosis even though the traits display fluctuating asymmetry (Palmer, 1994). Second, there could be intense natural or sexual selection against asymmetric individuals; hence, very asymmetric individuals may be relatively rare in field samples, if selection has already acted against the most asymmetric individuals. The platykurtic distribution of fluctuating asymmetry on the fore-leg is probably due to frequent leg regeneration after an autotomy event in early instars, which can blur the true fluctuating asymmetry of the leg.

The reason for the lack of correlations between fluctuating asymmetry among the different traits could be due to the fact that the traits have different developmental windows of vulnerability and that their development is controlled by different gene complexes (Parsons, 1990). Stresses may be specific to particular metabolic pathways and may not affect fluctuating asymmetry in all traits (Parsons, 1990).

Fluctuating asymmetry between sub-species

The reason why the amphigonic females of the sub-species *redtenbacheri* are more asymmetrical than the amphigonic females of the sub-species *rossius* is unclear, but it may be related to differences in the mean heterozygosity of the two sub-species. This would support the hypothesis that differences in fluctuating asymmetry are related to genetic diversification. Several studies have found a negative relationship between fluctuating asymmetry and protein heterozygosity, especially in poikilotherms. These types of organisms are perhaps more susceptible to temperature fluctuations, which may influence biochemical pathways associated with developmental and metabolic processes (Mitton, 1995; Møller and Swaddle, 1997). However, interpretation of these results must be made with caution, as Chakraborty (1987) has shown that heterozygosity at a few allozyme loci does not provide an accurate estimate of an individual's genomic heterozygosity.

The greater fluctuating asymmetry found in the parthenogenetic females of the sub-species *redtenbacheri* compared with the sub-species *rossius* cannot be due to differences in the mean heterozygosity of the two sub-species, as heterozygosity is zero in both sub-species. It could be the consequence of a different stress-susceptibility of the sub-species *redtenbacheri* as compared to *rossius,* or it could be that the *redtenbacheri* sample lived in a more stressed environment than the *rossius* sample.

Fluctuating asymmetry between reproductive systems within sub-species

It is clear from this study that the parthenogenetic females of both sub-species are much more asymmetric than the amphigonic females. Our results are in accordance with other authors' observations in that homozygous individuals are often less stable than their heterozygous counterparts (for example, Leary *et al.*, 1983, 1984; Clarke and McKenzie, 1987; but see Britten, 1996, for a different view). It is not surprising that parthenogenetic females with an almost complete homozygous genome are developmentally less stable than individuals

with a similar but more heterozygous genome. Furthermore, both parthenogenetic sub-species live in a degraded environment with vegetation made up of bramble shrubs, clearly a 'weedy' habitat (see Bullini and Nascetti, 1990), where the environmental fluctuations are expected to be higher than in the wealthier vegetation on which the amphigonic populations analysed are living. In fact, a degraded environment often displays a less structured ecosystem (Van Valen, 1965; Grant, 1967; Rothstein, 1973).

Fluctuating asymmetry between the sexes within sub-species

The greater fluctuating asymmetry of the males' antenna compared with that of the amphigonic females in both sub-species could be due to the length of the antenna being a sexually selected trait, following the suggestion that fluctuating asymmetry is higher in traits under directional selection (Møller and Thornhill, 1998). Møller and Swaddle (1997) suggested that directional selection will favour modifiers that decanalize the phenotype. However, Houle (1998) described seven distinct evolutionary processes that can affect genetic variance, and concluded that directional selection alone is not sufficient to favour decanalizing modifiers, which are favoured only when fitness rises faster than linearly with trait value (Lande, 1980). Another explanation could be that genes controlling antennal development are located on the sex chromosomes, for which the male sex is wholly hemizygous.

Morphological variance

Morphological variance between the two sub-species

We found evidence that the morphological variance of the parthenogenetic females of the *rossius* sub-species was greater than in the parthenogenetic females of the *redtenbacheri* sub-species. This is in contrast to the fluctuating asymmetry of the *redtenbacheri* parthenogenetic females being greater than that of the *rossius* sub-species. Developmental stability refers to the production of a specific phenotype under a given set of environmental conditions (Møller and Swaddle, 1997). Hence, we should expect that the more asymmetric parthenogenetic females of the *redtenbacheri* sub-species should also have greater morphological variance. These findings support the hypothesis that morphological variance is not a good predictor of developmental stability (see King, 1984) and support also Waddington's (1957) hypothesis that separate mechanisms are responsible for the effect of stress on trait variability and on developmental stability.

The lack of evidence of greater intrasexual morphological variance for males and amphigonic females of the *redtenbacheri* sub-species compared with the males and females of the *rossius* sub-species is contrary to our expectation. In fact, additive genetic variation may be sufficient to explain the phenotypic variation patterns among groups with different degrees of allozyme heterozygosity (Allendorf and Leary, 1986). However, these results must be interpreted with caution, as some allozyme loci do not provide an accurate estimator of an individual's genomic heterozygosity.

Morphological variance between the two reproductive systems within sub-species

The greater morphological variance of the amphigonic females compared with the parthenogenetic females in both sub-species can be explained by the fact that morphological variance

in parthenogenetic females is mainly due to environmental components, whereas that of amphigonic females is of both genetic and environmental origin. Morphological variance in a sexually reproducing population ($V_g > 0$) is given by: $V_p = V_g + V_{env} + (gxe) + cov(ge) + DI$, where (gxe) is the genotype by environment interaction, cov(ge) is the covariance between the genotypic and environmental source of variance and DI is developmental instability. The interaction term expresses the extent to which genotypic variants differ in their sensitivity to environmental effects. The cov(ge) has long been recognized as a confounding source of experimental error (for instance, when the fastest-growing animals are given the best diet). The term cov(ge) probably contributed to the increased morphological variance and increased developmental instability. The result obtained contrasts with Blum's (1988) hypothesis that stressful conditions may increase morphological variance. Under stressful conditions, minor changes in the environment may have large effects on traits, whereas they may have little impact in a more favourable environment.

Morphological variance between sexes within sub-species

The clearly higher morphological variance of the amphigonic females compared with males in the sub-species *rossius* could be due to the males' body size and its components (mesonotum, metanotum and abdomen) being the target of sexual selection, which reduces the morphological variance of the traits (Bulmer, 1985). The pattern observed in the sub-species *rossius* was the same in *redtenbacheri*, except for one highly significant contrasting result (metanotum) which is difficult to explain. However, Møller and Swaddle (1997) described several works where directional selection has been associated with increased morphological variation, supporting their hypothesis that directional selection will favour modifiers that decanalize the phenotype.

Lerner's conjecture

Lerner's conjecture predicts that individuals that have values for quantitative traits close to the mean of the population should also have the most symmetrical traits. Why we did not find such a pattern could be that body size in the two sub-species *rossius* and *redtenbacheri* is a sexually selected trait and, therefore, is under directional selective forces that could have confounded the expected pattern, or more simply the relationship between functional asymmetry and heterozygosity is too weak or absent. This finding contradicts the suggestion of Soulé and Cuzin-Roudy (1982) that homozygous individuals are less developmentally stable, but agrees with the results of Woods *et al.* (1999).

Future directions

We have provided evidence of greater development instability in the parthenogenetic individuals of two sub-species of *Bacillus rossius* compared with amphigonic individuals, which may be explained by a relatively higher cost of parthenogenetic reproduction compared to sexual reproduction. The higher buffering capacity of the amphigonic populations may be envisaged from a biochemical-genetic point of view: when different allelic products have different optima along a range of environmental conditions, heterozygotes will have the most stable genotype (see Schwartz and Laughner, 1969). When using morphological

variance as a precise estimator of developmental instability, populations should be genetically homogeneous ($V_g = 0$) and should not exhibit variation in environmental conditions across the habitat range of species ($V_{env} = 0$); however, these conditions are rarely fulfilled. Furthermore, if a trait is highly canalized and the peak of the size-fitness function is very narrow, there will be a small range in trait size variance between populations and differences will be difficult to detect. The results of several studies of the relationship between functional asymmetry and loss of genetic diversity have been controversial, but that could be due to a threshold relationship between functional asymmetry and genetic diversity (see Gilligan *et al.*, 2000). A clearer picture is required of the extent to which functional asymmetry is genetically based, as this is still an open debate (Møller and Thornhill, 1997; for criticisms, see Leamy, 1997; Markow and Clarke, 1997; Whitlock and Fowler, 1997). This is a fundamental point, because for functional asymmetry to have an evolutionary significance, it must have a heritable basis. A non-zero heritability is also a prerequisite for some hypotheses linking functional asymmetry to genetic stress (Pomiankowski, 1997). It is possible that non-additive genetical effects (dominance and epistatic interactions of genes) are an important feature in the genetical control of developmental stability (Leamy, 1984; Livshits and Kobyliansky, 1991). However, our study was of parthenogenetic individuals found in degraded environments. In a variable environment, the cost of clonal reproduction is high (Maynard Smith, 1978). Therefore, further studies should address other organisms in which parthenogenetic reproduction takes place under optimal conditions and sexual reproduction takes place under sub-optimal conditions. Similarly, it would be interesting to compare parthenogenetic and amphigonic individuals in species with different types of parthenogenesis where heterozygosity is maintained.

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REFERENCES

Allendorf, F.W. and Leary, R.F. 1986. Heterozygosity and fitness in natural populations of animals. *Conservation Biology*: *The Science of Scarcity and Diversity* (M.E. Soulé, ed.), pp. 57–76. Sunderland, MA: Sinauer Associates.

Barton, N.H. and Charlesworth, B. 1998. Why sex and recombination? *Science*, **281**: 1986–1990.

Blum, A. 1988. *Plant Breeding for Stress Environments.* Boca Raton, FL: CRC Press.

Britten, B.H. 1996. Meta-analyses of the association between multilocus heterozygosity and fitness. *Evolution*, **50**: 2158–2164.

Bullini, L. and Nascetti, C. 1990. Speciation by hybridization in phasmids and other insects. *Can. J. Zool.*, **68**: 1747–1770.

Bulmer, M.G. 1985. *The Mathematical Theory of Quantitative Genetics.* Oxford: Clarendon Press.

Chakraborty, R. 1987. Biochemical heterozygosity and phenotypic variability of polygenics traits. *Heredity*, **59**: 19–28.

Clarke, G.M. 1993. Patterns of developmental stability in *Chrysopa perla* L. in response to environmental pollution. *Environ. Entomol.*, **22**: 1362–1366.

Clarke, G.M. 1997a. Developmental stability and fitness: The evidence is not quite clear. *Am. Nat.*, **152**: 762–766.

Clarke, G.M. 1997b. The genetic basis of developmental stability. III. Haplo-diploidy: Are males more unstable than females? *Evolution*, **51**: 2021–2028.

Clarke, G.M. and McKenzie, J.A. 1987. Developmental stability of insecticide resistant phenotypes in blowfly: A result of canalizing natural selection. *Nature*, **325**: 345–346.

Clarke, G.M., Brand, G.W. and Whitten, M.J. 1986. Fluctuating asymmetry: A technique for measuring developmental stress caused by inbreeding. *Aust. J. Biol. Sci.*, **39**: 145–153.

Clarke, G.M., Oldroyd, B.P. and Hunt, P. 1992. The genetic basis of developmental stability in *Apis mellifera*: Heterozygosity *vs* genetic balance. *Evolution*, **46**: 753–762.

Crespi, B.J. and Brett, A.V. 1997. Fluctuating asymmetry in vestigial and functional trait of a haplodiploid insect. *Heredity*, **79**: 624–630.

Fowler, J. and Cohen, L. 1990. *Practical Statistics for Field Biology*. Philadelphia, PA: Open University Press.

Gilligan, D.M., Woodworth, L.M., Montgomery, M.E., Nurthen, R.K., Briscoe, D.A. and Frankham, R. 2000. Can fluctuating asymmetry be used to detect inbreeding and loss of genetic diversity in endangered populations? *Anim. Conserv.*, **3**: 97–104.

Grant, P.R. 1967. Bill length variability in birds of the Tres Marias Islands, Mexico. *Can. J. Zool.*, **45**: 805–815.

Haldane, J.B.S. 1955. The measurement of variation. *Evolution*, **9**: 484.

Hoffmann, A.A., Woods, R.E. and Clarke, G.M. 1999. A comment on ingroup–outgroup comparisons for fluctuating asymmetry based on trait values from the left or right sides of an individual. *Am. Nat.*, **153**: 140–142.

Houle, D. 1998. How should we explain variance in the genetic variance of traits? *Genetica*, **102/103**: 241–253.

Hurst, D.L. and Peck, J.R. 1996. Recent advances in understanding of the evolution and maintenance of sex. *TREE*, **11**: 46–52.

Imasheva, A.G., Loeschcke, V., Zhivotovsky, L.A. and Lazebny, O.E. 1997. Effects of extreme temperatures on phenotypic variation and developmental stability in *Drosophila melanogaster* and *Drosophila buzzatii*. *Biol. J. Linn. Soc.*, **61**: 117–126.

King, D.P.F. 1984. Enzyme heterozygosity associated with anatomical character variance and growth in the herring *Clupea harengus*. *Heredity*, **54**: 289–296.

Lande, R. 1980. Genetic variation and phenotypic evolution during allopatric speciation. *Am. Nat.*, **116**: 463–479.

Leamy, L. 1984. Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. *Am. Nat.*, **123**: 579–593.

Leamy, L. 1997. Is developmental stability heritable? *J. Evol. Biol.*, **10**: 21–29.

Leary, R.F., Allendorf, F.W. and Knudsen, K.L. 1983. Developmental stability and enzyme heterozygosity in rainbow trout. *Nature*, **301**: 71–72.

Leary, R.F., Allendorf, F.W. and Knudsen, K.L. 1984. Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. *Am. Nat.*, **124**: 540–551.

Lerner, I.M. 1954. *Genetic Homeostasis.* London: Oliver & Boyd.

Livshits, G. and Kobyliansky, E. 1984. Comparative analysis of morphological traits in biochemically homozygous individuals from a single population. *J. Hum. Evol.*, **13**: 161–171.

Livshits, G. and Kobyliansky, E. 1991. Fluctuating asymmetry as a possible measure of developmental homeostasis in humans: A review. *Human Biol.*, **63**: 441–466.

Mantovani, B. and Scali, V. 1991. Allozymic characterization of Sardinian *Bacillus rossius* (Rossi) and *B. atticus* Brunner (Insecta, Phasmatodea). *Genetica*, **83**: 275–287.

Markow, T.A. and Clarke, G.M. 1997. Meta-analysis of the heritability of developmental instability: A giant step backward. *J. Evol. Biol.*, **10**: 31–37.

Maynard Smith, J. 1978. *The Evolution of Sex.* Cambridge: Cambridge University Press.

Miller, R.G. 1981. *Simultaneous Statistical Inference.* New York: McGraw-Hill.
Mitton, J.B. 1995. Enzyme heterozygosity and developmental stability. *Acta Theriol.*, suppl. **3**: 33–54.
Møller, A.P. and Swaddle, J.P. 1997. *Asymmetry, Developmental Stability and Evolution.* Oxford: Oxford University Press.
Møller, A.P. and Thornhill, R. 1997. A meta-analysis of the heritability of developmental stability. *J. Evol. Biol.*, **10**: 1–16.
Møller, A.P. and Thornhill, R. 1998. Bilateral symmetry and sexual selection: A meta-analysis. *Am. Nat.*, **151**: 174–192.
Nascetti, G. and Bullini, L. 1983. Differenziamento genetico e speciazione in fasmidi dei generi *Bacillus* e *Clonopsis* (Cheleutoptera, Bacillidae). In *Atti XII Congresso Nazionale Entomologia*, pp. 215–223. Rome: Tipografia Sticca.
Palmer, A.R. 1994. Fluctuating asymmetry analyses: A primer. In *Developmental Instability: Its Origins and Evolutionary Implications* (T.A. Markow, ed.), pp. 335–364. Dordrecht: Kluwer.
Palmer, A.R. 1996. Waltzing with asymmetry. *BioScience*, **46**: 518–532.
Palmer, A.R. 1999. Detecting publication bias in meta-analyses: A case study of fluctuating asymmetry and sexual selection. *Am. Nat.*, **154**: 230–233.
Palmer, A.R. and Strobeck, C. 1986. Fluctuating asymmetry: Measurement, analysis, patterns. *Ann. Rev. Ecol. Syst.*, **17**: 391–421.
Parsons, P.A. 1990. Fluctuating asymmetry: An epigenetic measure of stress. *Biol. Rev.*, **65**: 131–145.
Pijnacker, L. 1969. Automictic parthenogenesis in the stick insect *Bacillus rossius* Rossi (Cheleutoptera, Phasmidae). *Genetica*, **40**: 393–399.
Pomiankowski, A. 1997. Genetic variation in fluctuating asymmetry. *J. Evol. Biol.*, **10**: 51–55.
Rice, W.R. 1989. Analysing tables of statistical tests. *Evolution*, **43**: 223–225.
Rothstein, S.I. 1973. The niche-variation model. Is it valid? *Am. Nat.*, **107**: 598–620.
Rutherford, S.L. and Lindquist, S. 1998. HSP90 as a capacitor for morphological evolution. *Nature*, **396**: 336–342.
Scali, V. 1969. Osservazioni citologiche sullo sviluppo embrionale di *Bacillus rossius*. *Acc. Naz. Lincei Rend. Cl. Sc. Fis. Mat. Nat., Serie VIII*, **46**: 110–116.
Scali, V. 1996. Distribution, genetic diversification and reproductive mechanisms of unisexual *Bacillus* stick insects. In *Abstracts of the Fifth International Congress of Systematic and Evolutionary Ecology*, p. 165. Budapest: Pars Ldt.
Scali, V. and Mantovani, B. 1989. Updating of systematics and speciation mechanisms of *Bacillus* (Insecta, Phasmatodea). *Boll. Zool.*, **56**: 87–98.
Schwartz, D. and Laughner, W.J. 1969. A molecular basis for heterosis. *Science*, **166**: 626–627.
Soulé, M.E. and Cuzin-Roudy, J. 1982. Allometric variation. II. Developmental instability of extreme phenotypes. *Am. Nat.*, **120**: 765–786.
Thoday, J.M. 1958. Homeostasis in selection experiments. *Hereditas*, **12**: 401–415.
Tinti, F. 1993. Morphological, genetic and chromosomal characterization of Corsican and Spanish *Bacillus rossius* (Insecta, Phasmatodea). *Vie Milieu*, **43**: 109–117.
Tinti, F., Mantovani, B. and Scali, V. 1992. Caratterizzazione allozimatica di popolazioni di *Bacillus rossius* dell'Italia centro-meridionale e della Sicilia (Insecta, Phasmatodea). *Boll. Soc. It.* (*Museo Genova*), **123**: 184–194.
Van Valen, L. 1965. Morphological variation and the width of the ecological niche. *Am. Nat.*, **99**: 377–390.
Waddington, C.H. 1957. *The Strategy of the Genes.* New York: Macmillan.
Wagner, G.P., Booth, G. and Bagheri-Chaichian, H. 1997. A population genetic theory of canalization. *Evolution*, **51**: 329–347.
White, M.J.D. 1973. *Animal Cytology and Evolution*, 3rd edn. Cambridge: Cambridge University Press.
Whitlock, M.C. and Fowler, K. 1997. The instability of studies of instability. *J. Evol. Biol.*, **10**: 63–67.

Williams, G.C. 1975. *Sex and Evolution.* Princeton, NJ: Princeton University Press.

Woods, R.E., Sgrò, C.M., Hercus, M.J. and Hoffmann, A.A. 1999. The association between fluctuating asymmetry, trait variability, trait heritability, and stress: A multiply replicated experiment on combined stresses in *Drosophila melanogaster*. *Evolution*, **53**: 493–505.

Zar, J.H. 1984. *Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice-Hall.

Zouros, E., Singh, M. and Miles, H. 1980. Growth rate in oysters: An overdominant phenotype and its possible explanation. *Evolution*, **34**: 856–867.