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Effects of bottlenecks on quantitative genetic variation in the butterfly *Bicyclus anynana*

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Summary

The effects of a single population bottleneck of differing severity on heritability and additive genetic variance was investigated experimentally using a butterfly. An outbred laboratory stock was used to found replicate lines with one pair, three pairs and 10 pairs of adults, as well as control lines with approximately 75 effective pairs. Heritability and additive genetic variance of eight wing pattern characters and wing size were estimated using parent–offspring covariances in the base population and in all daughter lines. Individual morphological characters and principal components of the nine characters showed a consistent pattern of treatment effects in which average heritability and additive genetic variance was lower in one pair and three pair lines than in 10 pair and control lines. Observed losses in heritability and additive genetic variance were significantly greater than predicted by the neutral additive model when calculated with coefficients of inbreeding estimated from demographic parameters alone. However, use of molecular markers revealed substantially more inbreeding, generated by increased variance in family size and background selection. Conservative interpretation of a statistical analysis incorporating this previously undetected inbreeding led to the conclusion that the response to inbreeding of the morphological traits studied showed no significant departure from the neutral additive model. This result is consistent with the evidence for minimal directional dominance for these traits. In contrast, egg hatching rate in the same experimental lines showed strong inbreeding depression, increased phenotypic variance and rapid response to selection, highly indicative of an increase in additive genetic variance due to dominance variance conversion.

1. Introduction

The role of population bottlenecks in adaptive evolution rests crucially on how genetic drift and inbreeding affect heritable variation of quantitative characters (Lande, 1980). In a purely additive model, with no selection, a bottleneck of N_e individuals reduces additive genetic variance V_A by a factor of $1/2N_e$, lowering the ability of the population to respond to selection (Wright, 1951; Falconer & Mackay, 1996). In reality, many polygenic characters deviate from additivity to some degree (reviewed in Moreno, 1994). In light of this, several theoretical studies have found that, under certain model assumptions, inbreeding can lead to a net increase (or $< 1/2N_e$ reduction) in V_A through the 'conversion' of non-additive genetic variance. This non-additive variance was hitherto unavailable to selection because it was in the form of dominance variance (Robertson, 1952; Willis & Orr, 1993), epistatic variance (Goodnight, 1987, 1988; Cheverud & Routman, 1996; López-Fanjul *et al.*, 1999) or both (Tachida & Cockerham, 1989; Whitlock *et al.*, 1993). However, the validity of these models for a given trait is highly dependent on its underlying genetic architecture (i.e.

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the relative magnitudes of additive, dominance and epistatic variances, as well as the distribution of allelic effects), which is, in general, unknown (Barton & Turelli, 1989). Moreover, it is difficult to predict whether selection will act unconditionally against the novel genotypes produced by inbreeding, such that any increase in V_A is counterbalanced by a reduction in mean fitness, hence compromising its adaptive value.

The endurance of the debate on the likelihood of bottleneck-induced increases in V_A (e.g. Coyne *et al*., 2000; Goodnight & Wade, 2000) stems from the fundamental implications of such effects for founder event speciation (e.g. Barton & Charlesworth, 1984; Carson & Templeton, 1984; Barton, 1989; Carson, 1990; Templeton, 1996), Wright's shifting balance (e.g. Wright, 1977; Whitlock, 1995) and the evolutionary consequences of metapopulation dynamics (Lande, 1992; Harrison & Hastings, 1996). A fuller understanding of the effect of drift on polygenic variation is also of applied importance in the conservation of biodiversity (Frankham, 1999).

The debate has been fuelled by the results of laboratory experiments: some demonstrate close concordance to the neutral additive model, while others have found significant deviations from this classical model. The most influential of these empirical studies was that of Bryant *et al*. (1986), followed up in Bryant & Meffert (1993), showing that V_A for some morphological characters of *Musca domestica* can increase substantially after bottlenecks. Bottleneck-induced increases in V_A have also been shown for sternopleural bristle number (Lints & Bourgois, 1984), viability (López-Fanjul & Villaverde, 1989; García *et al*., 1994) and courtship song (Ritchie & Kyriacou, 1994) in *Drosophila melanogaster*; and for viability (Fernández *et al*., 1995) and fitness (Ruano *et al*., 1996; Wade *et al*., 1996) in *Tribolium castaneum*. In contrast, some earlier experiments (Da Silva, 1961; James, 1971; Frankham, 1980; Franklin, 1980) found that bottlenecked lines of *Drosophila* exhibited a reduced short-term response to artificial selection on sternopleural or abdominal bristle number. Moreover, for a bottleneck of one breeding pair, the magnitude of the response was close to the expected three quarters of the response of non-bottlenecked lines, in all four experiments. More recently, Wade *et al*. (1996) found that the reduction in the realized heritability of pupal weight in differentially inbred lines of *T. castaneum* was of the order predicted by the neutral additive model. Similarly, Whitlock & Fowler (1999), on the basis of a highly replicated isofemale treatment, concluded that the change in V_A for *D. melanogaster* wing size and shape was consistent with neutral additive expectations.

In most of the cases presented above the observed pattern of response to inbreeding can be accounted for by variation in dominance variance for the traits under study (Willis & Orr, 1993). Increases in V_A are usually associated with characters showing inbreeding depression (Bryant *et al*., 1986; López-Fanjul & Villaverde, 1989; García *et al*., 1994; Fernández *et al*., 1995; Ruano *et al*., 1996; Wade *et al*., 1996), implying that dominance variance (as opposed to epistatic variance) is the major contributor to the increase. In contrast, studies which conform to the neutral additive model measured either V_A of bristle number in *Drosophila* (Da Silva, 1961; James, 1971; Frankham, 1980; Franklin, 1980), heritability of pupal weight in *T. castaneum* (Wade *et al*., 1996) or V_A of wing size and shape in *D. melanogaster* (Whitlock & Fowler, 1999). These characters show minimal inbreeding depression (e.g. Latter & Robertson, 1962; Kidwell & Kidwell, 1966; Goodwill, 1975), indicating no substantial directional dominance for these characters.

A notable exception to this trend is the absence of any significant reduction in V_A for adult body weight in mice inbred to an average coefficient of inbreeding of 0·39 (Cheverud *et al*., 1999). Given the minimal inbreeding depression for this trait, direct evidence for epistasis (from QTL data) and high level of experimental replication this is the most convincing example to date of inbreeding-induced epistatic to additive variance conversion. Loss of V_A may also be retarded by selection against homozygotes in combination with linkage disequilibrium (see Latter *et al*., 1995), as reported by Tantawy & Reeve (1956) who found that V_A for wing size in *D. melanogaster* showed no significant decline up to 50–60% inbreeding and remained higher than expected up to nearly 90% inbreeding.

The present experiment was conducted to investigate the effects of single generation bottlenecks (founder events) of different size on quantitative genetic variation of morphological characters – wing size and pattern – in the satyrine butterfly *Bicyclus anynana* (Butler). This butterfly has a wing pattern which is both highly variable and easily measured. Moreover, the wing pattern is known to be under strong visual selection in relation to attacks by vertebrate predators (Brakefield, 1997). Wing pattern diversity is also a feature of the speciose genus *Bicyclus*, consisting of about 80 species (Condamin, 1973). In nature, *B. anynana* populations are probably more stable and also more spatially substructured (Brakefield & Reitsma, 1991) than *Musca* or *Drosophila* populations, which are typically very large, yet prone to crashes, partly as a result of more ephemeral food resources and more rapid turnover of generations (e.g. Sevenster & van Alphen, 1993). The influence of demographic history and ecology on genetic architecture is complex (Roff, 2000), but is likely to have implications for the response to bottlenecks (see Saccheri *et al*., 1996).

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It is evident from the foregoing overview that, in order to critically assess the influence of genetic architecture on the response of V_A to bottlenecks, one should ideally (pre)select a suite of traits which vary substantially with respect to additive, dominance and epistatic variances. For practical reasons it was not possible to study the non-additive components of variance directly, though the magnitude of directional dominance was assessed. Furthermore, while the change in V_A was measured for morphological traits only, we also report on the likely genetic basis of the selection response of a major component of fitness (egg-larva viability) in the same experimental lines (Saccheri *et al*., 1996).

The aim of this experiment was to provide results which could be compared with previous empirical studies, particularly those of Bryant *et al*. (1986), and with the neutral additive model, using a novel model organism. A specific strength of this study is that the degree of inbreeding in the experimental lines was estimated directly with molecular markers (Saccheri *et al*., 1999), overcoming the difficulty of uncertain N_e encountered by some other bottleneck experiments. This paper describes the effects of the bottlenecks on heritability and additive genetic variance within lines for wing size and a series of wing pattern characters. Given the paucity of empirical data on this important issue, primarily on a few traits in four species (*D. melanogaster*, *M. domestica*, *T. castaneum* and *Mus musculus*), fresh data describing the response of very different traits in an unrelated species are of substantial interest for understanding the response of polygenic traits to inbreeding.

2. Materials and methods

(i) *Experimental populations*

A laboratory population of *B. anynana* was established from approximately 80 gravid females collected from the wild at a single locality (Nkhata Bay) in Malawi in August 1988. Prior to the experiment the stock was maintained for about 20 generations at a population size of 400–600 adults with some overlap of generations. High levels of allelic diversity and heterozygosity detected by single-locus DNA fingerprinting and allozyme electrophoresis (Saccheri *et al*., 1999) suggest that the stock population at the start of the experiment had not undergone any substantial bottleneck and that the husbandry technique was effective in maintaining genetic variability.

Previous experimental results (e.g. Bryant *et al*., 1986) and theoretical predictions suggest that the largest changes in additive genetic variance are most likely to occur for population bottleneck sizes (founder events) of 20 or fewer individuals. Consequently, daughter populations were derived from the base population according to four treatments, differing in the number of individuals used to start the experimental populations or lines. The founder numbers used were two, six, 20 and *c*. 300 (control treatment), replicated six times for the smallest bottleneck and four times for the rest.

Husbandry techniques are detailed in Saccheri *et al*. (1996). With the exception of the control lines (see below), all lines were established from clutches of known parentage in the following way. One hundred and twenty-five clutches were collected over 7 days from females isolated *in copula*. From those clutches with more than 40 fertile eggs (representing 80 % of all fertile clutches and 57 % of all clutches, including non-fertile ones) the required number of clutches (i.e. one, three or 10) were randomly chosen to found the bottlenecked populations of one pair, three pairs and 10 pairs. These populations were then allowed to freely increase in size to a maximum adult population size of about 300, controlled by random culling of larvae. Three generations elapsed before data were collected (population sizes from P to F3 are given in Saccheri *et al*., 1999).

It was impractical to establish control lines with clutches obtained from mating pairs collected *in copula*. However, to control for the small chance that the procedure for choosing clutches to found bottleneck lines was biased with respect to genotype, two types of control were used. Initially, four populations each consisting of 150 virgin females and 150 virgin males were established and random mating allowed for 2 weeks. For two of these populations we collected eggs from the entire population; these eggs were used to found two control lines. For the remaining two populations we collected clutches from all females individually, but only used those clutches with more than 40 fertile eggs (85 and 60 clutches from each population respectively) to found the other two control lines. In subsequent generations, all females were given equal opportunity to lay eggs on potted maize plants placed in the cages for a fixed period of time, all control lines being maintained at an adult population size of about 300. Clutches used to found the 10 pair and control lines were culled randomly to keep larval densities equivalent in all lines. No differences in inbreeding were detected between the two types of control (see Saccheri *et al*., 1999).

(ii) *Morphological measurements*

Butterflies to be measured were frozen and dissected to separate the wings from the body, which was required for molecular analysis (Saccheri *et al*., 1999). Nine morphological characters were measured on the ventral surface of the hind wing (Fig. 1): the areas of three concentric rings of colour constituting the fifth and largest of the eyespots occuring in the distal part of the wing (referred to as white area, black area and

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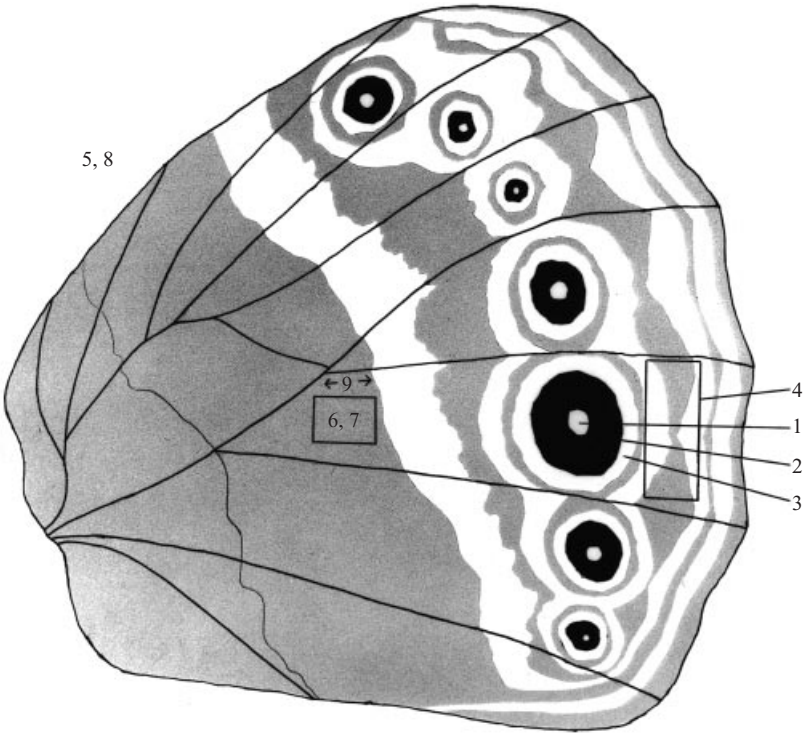

Fig. 1. Morphometric measurements of *Bicyclus anynana* hind wing.

No.	Object	Measurement	Character name
1	White centre spot	Area (mm²)	White area
2	Black ring	Area (mm²)	Black area
3	Gold ring	Area (mm²)	Gold area
4	Extra ring	Standard deviation of grey values	Extra ring
5	Whole wing	Coarse grey value differences	Contrast
6	Mid-cell	Mean grey value (small = dark)	Colour
7	Mid-cell	Fine grey value differences	Marbling
8	Whole wing	Area (mm²)	Wing area
9	Mid-cell	Distance from crossing point of cubital and median veins to edge of white band (mm); inversely proportional to band area	Band index

gold area); four indices of colour and contrast over different parts of the wing surface, measured on a scale of grey values ranging from 0 to 255 (extra ring, colour, contrast and marbling); the area of the entire wing (wing area); and an index of the area of the white transverse band (band index). The left hind wing was measured unless damage necessitated measurements from the right hind wing. All traits were measured with a computerized image analyser developed specifically for this purpose (Windig, 1993). Repeated measurements of the nine morphological traits for a subsample of 100 males yielded estimates of repeatability from 0·697 to 0·936 with a mean of 0·852. The two traits associated more with wing colour and variation in grey values (colour and marbling) showed lower repeatability than the size and pattern traits.

The nine morphological traits which were measured are not all developmentally independent. This is most obvious for the three eyespot components (Brakefield, 2000). Principal component analysis was used to summarize the variation contained in the nine morphological characters, while at the same time providing principal components which could be treated as statistically independent. The raw character values were transformed into standardized normal deviates as part of the analysis, which was performed in Genstat (Payne *et al*., 1988). A correlation matrix, as opposed to a covariance matrix, was used because of the widely differing scales of measurement and units.

(iii) *Heritability tests*

Parent–offspring heritability tests were carried out for the base population at the start of the experiment (test 1) and all 18 lines in the F3 generation (test 2). Single male–female pairs were isolated and their offspring reared under the same conditions of temperature and humidity. Clutches were culled to standardize larval

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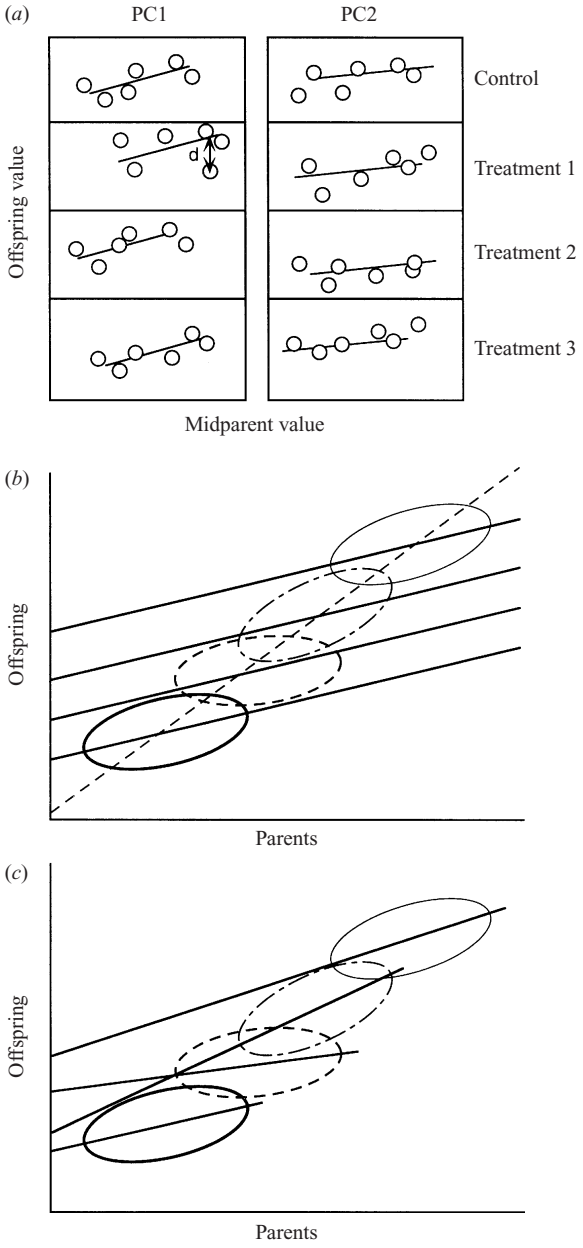

Fig. 2. Diagrams to clarify our analysis and estimation procedures. (*a*) Under the null hypothesis we expect no significant difference in the slopes of parent–offspring regressions (i.e. heritability) among treatments (only one replicate with six families is shown per treatment), though slopes may differ among principal components. (*b*) The heritability for a particular treatment can be estimated by fitting parallel regression lines, with a different intercept for each of the four (or more) replicates. Failure to fit different intercepts would result in the upwardly biased estimate shown by the dashed line. (*c*) The full model fits different slopes for each replicate within a treatment (i.e. different heritabilities).

density in the sleeve cages, which were kept abundantly supplied with maize. In test 1, 57 families were reared; both parents, 10 daughters and 10 sons were measured for each family. In test 2, an average of 12 families per line were reared giving treatment totals of 63, 55, 51 and 49 families for one pair, three pair, 10 pair and control treatments respectively; both parents, five daughters and five sons were measured for each family. Heritability, h^2, was estimated from the slope of the regression of offspring on parental values within each line. Additive genetic variance, V_A, was estimated as the product of the midparent heritability and twice the phenotypic variance, V_{MP}, of 30 mid-pair values (parents plus randomly chosen pairs from the same line) for each line. The environmental variance, V_E, was calculated as $2V_{MP}-V_A$.

(iv) *Inbreeding depression*

The extent of any reduction in the mean value of a trait following inbreeding (i.e. inbreeding depression) is a measure of the magnitude of directional dominance for that trait. ANOVA was used to test for equality of treatment (bottleneck size) means for all nine morphological traits in each sex of the parental F3 generation sample (30 individuals of each sex per line). Statistical significance was assessed by comparing the ratio of treatment to replicate (nested within treatment) mean squares with the appropriate critical value of the *F*-distribution.

(v) *Analysis*

The experiment was designed to evaluate the effect of bottleneck treatments on h^2, which can be divided into effects on V_A and on V_E. The neutral additive model makes specific predictions which can be compared with the observed results. The rationale for the analysis was as follows. The slope of the regression for the trait in offspring on parents is an estimate of h^2. Under the null hypothesis that treatment had no effect, the parent–offspring regressions should not differ across treatments for each principal component (Fig. 2*a*). Given a normal distribution, the likelihood for a particular regression line is a function of deviation of each offspring value from the regression line, d, and the variance, V:

$$\text{Log likelihood} = -0{\cdot}5\Sigma \ln(V) + d^2/V,$$

where the sum is over all offspring. The likelihood can therefore be maximized by minimizing the sum of squares of deviations, and likelihoods can be combined over the principal components if each regression is weighted by its residual deviance.

Although principal components are constructed so that they are uncorrelated (Manly, 1986), they may not be independent within a line after the bottleneck. This is because linkage disequilibrium will have been generated by the bottlenecks (Lynch, 1988), with the result that changes in h^2 and V_A for different principal components within a line may be associated. This would have the effect of inflating the variation in slope

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Table 1. *Estimates of the coefficient of inbreeding at F3, used to calculate the expected heritability and additive genetic variance for each bottleneck size, according to three series of estimates* (*see text*)

Bottleneck size	*E*(null)	*E*(demographic)	*E*(molecular)
One pair	0	0·27	0·32
Three pairs	0	0·10	0·12
Ten pairs	0	0·03	0·07
Control	0	0·01	0·02

between replicates (measured by the *E*xpectation × bottleneck size × replicate term in Tables 4–6) and reducing it between principal components (pc) of the same replicate (*E*xpectation × replicate × pc). In fact the mean squares for these two terms were very similar, but it might be considered prudent to interpret the data using the former term as the denominator in *F*-tests. Correlated changes for different principal components within lines may also reflect variation in inbreeding among replicate lines within treatments, but the precision of our estimates for h^2 is insufficient to study the behaviour of individual replicates.

Each treatment (bottleneck size) slope (heritability) was estimated by fitting a common slope to all replicate lines of the treatment, while allowing for different intercepts for each replicate. In effect, this procedure fitted a set of parallel straight lines (four or six per treatment) through the data (Fig. 2*b*). This estimation procedure is preferable to taking the mean slope because the precision of the estimates differs between replicates. Fitting parallel lines takes this into account automatically. Notice that this approach is not equivalent to pooling the data across replicates, which would lead to upwardly biased h^2 estimates due to differentiation among replicate line means (Fig. 2*b*).

A test for deviation from parallel regression lines (Fig. 2*a*) was conducted by fitting a different slope to each treatment and testing for a significant improvement in fit (*E*xpectation × bottleneck size in Tables 4–6). Any improvement in fit produced by allowing a different slope for each replicate (Fig. 2*c*) quantifies the different response of the replicates of each treatment (*E*xpectation × bottleneck size × replicate in Tables 4–6). Terms were also introduced to quantify the different response of principal components within replicates (*E*xpectation × replicate × pc in Tables 4–6). A more stringent null hypothesis is that the ratio of slopes for each principal component is predicted by the neutral additive model and the effective population size N_e of each bottleneck. In that case the regressions were transformed to be parallel using the procedure described below. Deviation from the null expectation was tested in the same way by fitting a separate slope for each treatment, and again information was combined across principal components by weighting each regression by its residual deviance.

If one assumes that the morphological characters have no non-additive variance, as is reasonable based on our observations implying small dominance variance, and that environmental variance is unaffected by inbreeding, the expected heritability after inbreeding is given by

$$E(h_t^2) = \frac{h_0^2(1-F_t)}{(1-h_0^2F_t)}$$

where $E(h_t^2)$ and F_t are the heritability and inbreeding coefficient at time t, and h_0^2 is the original heritability in the base population (Falconer & Mackay, 1996). For the analysis of heritability, the expected deviation of offspring values from the mean was calculated as

$$(\text{midparent value–line mean}) \cdot E(h_t^2),$$

for each principal component. $E(h_t^2)$ is the ratio of the expected additive variance, $E(V_A)$, and the expected midparental phenotypic variance, $E(V_{MP})$. A second approach attempted to account for differences in environmental variance between replicates in order to focus on the response of V_A. In this analysis the expected deviation of offspring values from the mean was calculated as

$$(\text{midparent value–line mean}) \cdot E(h_t^2) \cdot \frac{E(V_{MP})}{V_{MP}},$$

where $E(V_{MP}) = V_{A0}(1-F_t) + V_{E0}$, V_{E0} being the additive and environmental variances in the base population, respectively. The observed heritabilities and phenotypic variances for the base population were not substantially different from the control averages, but as the base population was reared under slightly different conditions, the control estimates were used for h_0^2 and V_{MP0}.

Three different expectations, referred to as *E*(null), *E*(demographic) and *E*(molecular), were specified by different estimates of F_t for each bottleneck size (Table 1). *E*(null) is equivalent to the null hypothesis that the bottlenecks have no effect; *E*(demographic) are the null expectations from the calculation that

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includes inbreeding due to observed population sizes and variances in family size in the F1 and F2 (see Saccheri *et al*., 1996); and *E*(molecular) uses the estimates obtained from the analysis of allozyme and minisatellite data (Saccheri *et al*., 1999). The control data were included in each analysis, with F_t set to zero. The estimated slope for control values against their expectation need not be zero. This scheme allowed for error in the estimation of h_0^2 and V_{MP0} which could otherwise lead to a systematic deviation from expectations across all the treatments.

3. Results

(i) *Principal component analysis*

Plots of the cumulative frequency distribution of each character against the cumulative normal were linear, indicating that the characters are normally distributed. A principal component analysis was carried out on all the test 2 offspring (1042 females and 985 males), these being in a better preserved state than the parent generation sample (Table 2). The first three principal components can each be interpreted in a straightforward way: PC1 (which explains 31 % of the total variation) is essentially an index of eyespot size, large eyespots being associated with high values of extra ring and contrast; PC2 (21 %) is essentially an index of darkness and grain in the mid-cell; and PC3 (13 %) contrasts wing area and band area. PC4, PC5 and PC6 are more difficult to interpret, but are consistent with other data sets and each explains between 5 % and 10 % of the total variation.

Table 2. *Principal component character loadings*

	Principal component					
Character loadings	1	2	3	4	5	6
White area	−0·445	−0.198	−0·035	0·044	0·358	−0·225
Black area	−0·437	−0·298	0·097	0·098	0·281	−0·064
Gold area	−0·480	−0·213	0·056	0·123	0·086	0·188
Extra ring	−0·374	0·340	−0·323	−0·400	−0.136	−0·110
Contrast	−0·428	0·227	−0·043	−0·312	−0·475	0·090
Colour	−0·148	0·507	0·371	0·198	0·222	0·664
Marbling	−0·107	0·507	0·387	0·335	−0·013	−0·664
Wing area	−0·107	−0·375	0·507	0·134	−0·642	0·033
Band index	0·111	−0·069	0·580	−0·739	0·284	−0·087
Eigenvalue (%)	32	21	13	10	10	5

The eigenvalues (as percentages) and eigenvectors of the correlation matrix for the nine wing characters measured in *B. anynana*. The first six principal components are shown; the largest loadings for each principal component are underlined. Note that band index is inversely proportional to band area.

Table 3. *Analysis of covariance from the GLM of offspring values with the midparental values as the explanatory variate* (*control only*)

Source of variation	d.f.	s.s.	m.s.	v.r.	P^a
Offspring sex × replicate[b] × pc	47	281·055	5·980	7·67	*
Try[c] parent sex × offspring sex	4	428·032	107·008	137·33	***
Midparent	1	425·013	425·013	545·45	***
Midparent × pc	5	15·358	3·072	3·94	ns
Midparent × replicate	3	2·761	0·920	1·18	ns
Midparent × replicate × pc	15	55·394	3·693	4·74	ns
Replicate × pc × family	246	757·457	3·079	3·95	*
Residual	2574	2005·671	0·779		
Total	2891	3542·708	1·225		

* $0{\cdot}01 < P \leqslant 0{\cdot}05$; ** $0{\cdot}001 < P \leqslant 0{\cdot}01$; *** $P \leqslant 0{\cdot}001$; ns, non-significant.

[a] Levels of significance are calculated in relation to the m.s. for replicate × pc × family as the denominator (with the exception of the *P* value for replicate × pc × family, where the residual m.s. is the denominator). *P* value for offspring sex × replicate × pc refers to the separate effects of replicate and pc, which were the only factors in this group that differed significantly in their means.

[b] Replicate stands for replicate within treatment.

[c] Try is an option in generalized linear modelling which adds terms then drops them.

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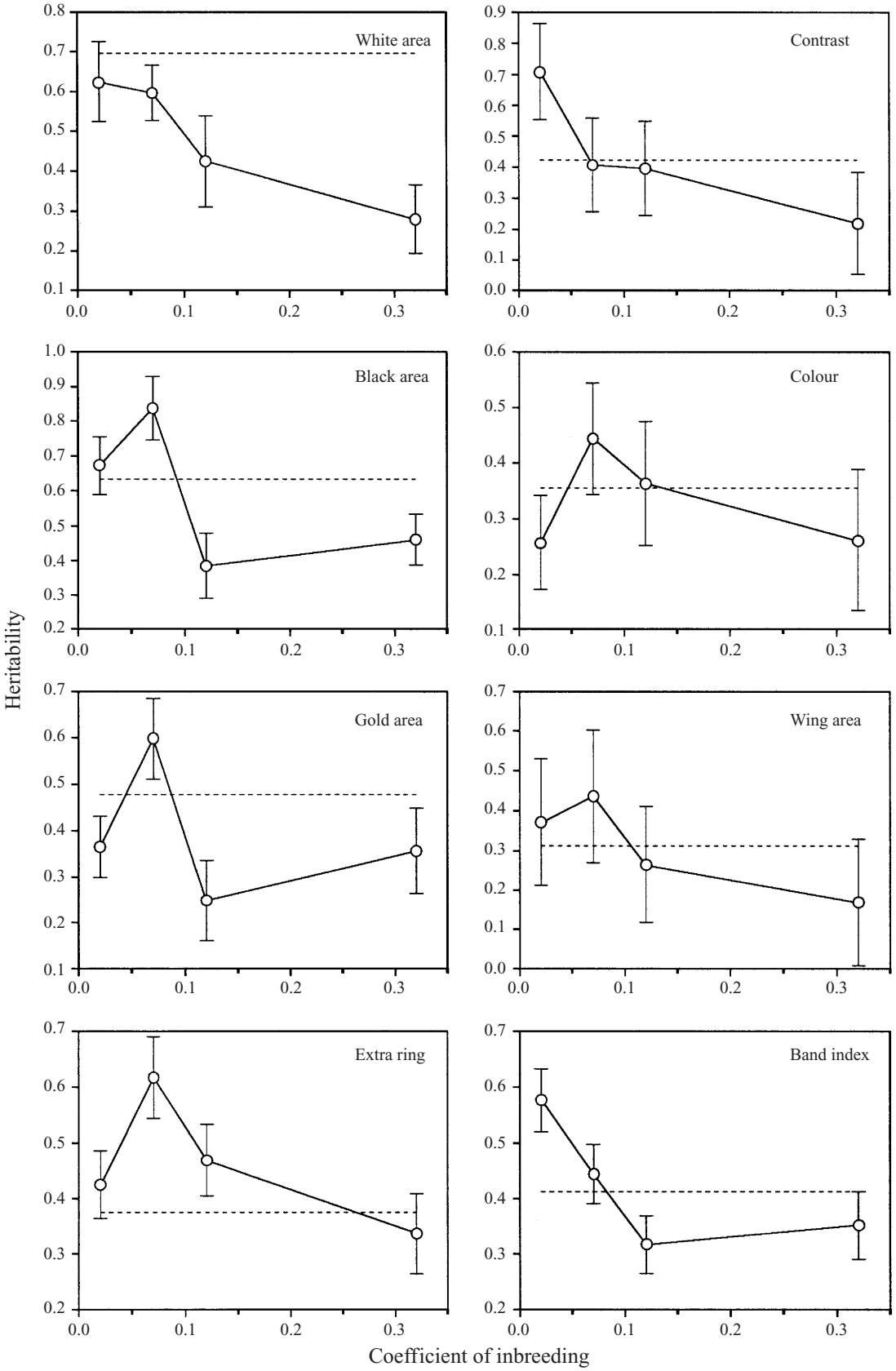

Fig. 3. Narrow-sense heritabilities for eight morphometric traits determined by offspring–midparent regressions for bottleneck lines of one, three and 10 founding pairs and control lines (continuous line). Average heritabilities for each bottleneck size and their standard errors were calculated by fitting the best set of parallel regressions to replicate lines within each bottleneck size (six for the one pair and four for the other bottleneck sizes). These average heritabilities are

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(ii) *Inbreeding depression*

There was no detectable inbreeding depression for any of the morphological traits and, by extension, for the principal components. The one significant result ($P = 0{\cdot}04$) was for male wing area. However, the pattern of line and treatment means shows that this result, which is primarily due to two low three pair lines, cannot be accounted for by inbreeding depression. Such a lack of evidence for directional dominance also suggests little dominance variance in general for these traits.

(iii) *Heritability, additive genetic variance and environmental variance*

An initial exploratory analysis of principal component heritabilities was performed on the four control lines (Table 3). There was a significant common family effect (replicate × pc × family), so mean squares of previous terms were compared with the 'family' mean square to assess their level of statistical significance. Separate intercepts were first fitted for sons and daughters (offspring sex), replicate lines and each of the six principal components. Only replicates and principal components differed significantly in mean (remembering that female and male values were standardized), as might be expected due to environmental variance between cages and the differing character composition of principal components. The pooled sums of squares obtained by fitting a different slope for each parent sex and their interaction with each offspring sex is not significantly larger than the sum of squares for the regression of all offspring on midparent values. This result indicates that h^2 do not differ significantly between parent or offspring sex and validates the use of midparent h^2 in subsequent analyses. Somewhat surprisingly, principal components do not appear to differ significantly with respect to their h^2 (midparent × pc). There were no significant differences in h^2 among the four replicates (midparent × replicate), or any significant interaction between replicates and principal components with respect to h^2 (midparent × replicate × pc).

Character heritabilities are not independent and cannot therefore be combined directly, but are shown to illustrate the results in their simplest form (Fig. 3). In general, there is a clear tendency for the one pair and three pairs bottleneck size lines to have lower h^2 than the 10 pairs and control bottleneck lines. The raw character V_A graphs (Fig. 4) show a pattern of treatment effects similar to that observed for h^2: an increasing trend from the one pair and three pair bottlenecks to the 10 pair bottleneck and control.

Differences between base population and F3 control h^2 are most likely to be due to differences in the rearing environment resulting from higher larval densities in the test 1 parental generation. The closer correspondence between base population and control F3 h^2 for area measures (white area, black area, gold area and wing area), as opposed to grey value measures (extra ring, contrast and colour), suggest that the former characters are the most reliable, at least in part because they have higher repeatabilities and are less affected by wing wear.

The analyses of effects on h^2 and V_A in relation to the null hypothesis are presented in Tables 4 and 5, respectively. The variation between replicates is not significantly greater than the variation between principal components within replicates. It is not clear, therefore, whether the treatment effect should be compared with the mean square of summing all the terms of $E \times$ bs × replicate not already included in the model, or with the $E \times$ replicate × pc mean square (i.e. the pooled sums of squares). Sokal & Rohlf (1981, p. 284) state that 'statisticians do not agree about the conditions under which mean squares should be pooled and about the desirability of pooling' in such nested models. They propose criteria under which pooling should be performed. According to these, the sums of squares should be pooled in this analysis if the prior belief was that the variance among replicates was less than twice that among principal components. This might be applicable in this case where the principal components are considered to reflect different traits. The increased number of degrees of freedom changes the significance level of the results as indicated in parentheses in Tables 4 and 6, but relies on the assumption of independence between principal components.

Bottleneck size changed h^2 and V_A significantly (E(null) × bs in Tables 4 and 5). This result holds whether the treatment effect is compared with the mean square of $E \times$ bs × replicate or $E \times$ replicate × pc, but is more convincing in the latter case. The pooled sums of squares for E(null) and E(null) × bs is larger (though not significantly) in Table 4 (h^2 analysis) than in Table 5 (V_A analysis). This suggests that the second approach, using V_{MP}, is a less efficient predictor of offspring values, implying either that the error on V_{MP} estimates is too large for them to be used to adjust h^2, or that the assumption that variation in V_{MP} was predominantly due to environmental variation was inappropriate.

The analyses investigating whether the observed changes in h^2 and V_A deviated significantly from the changes expected under the neutral additive model,

plotted against the treatment level coefficient of inbreeding estimated from demographic and molecular data combined (Saccheri *et al*., 1999). Base population values (dashed line) are shown for comparison. The graph for marbling is similar to that for colour with which it is correlated, and is not shown.

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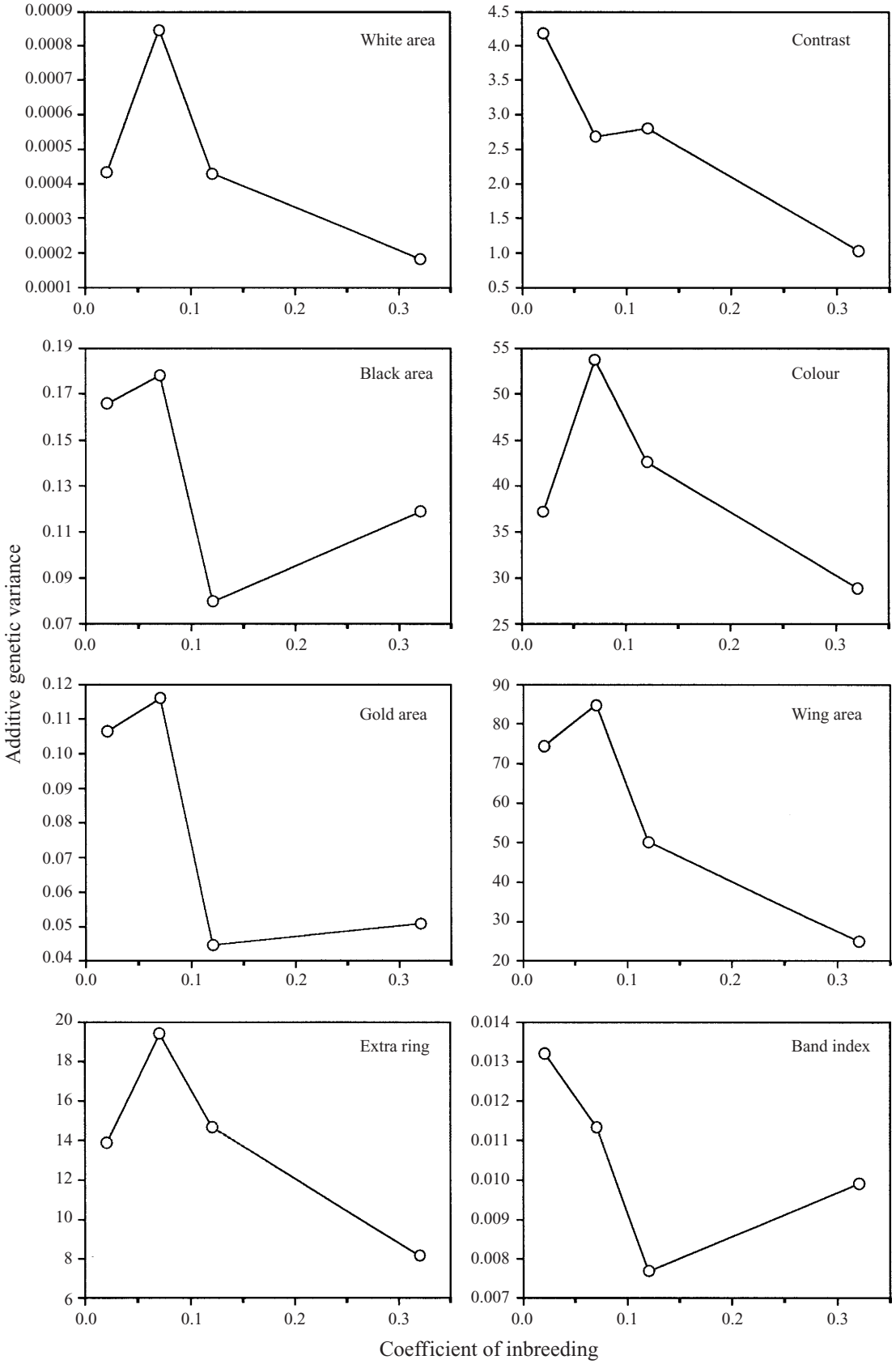

Fig. 4. Additive genetic variances for eight morphometric traits averaged across replicate lines within each bottleneck size, plotted against the treatment level coefficient of inbreeding.

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Table 4. *Analysis of covariance from the GLM of offspring values with the midparental values as the explanatory variate* (*all treatments*)

Source of variation	d.f.	s.s.	m.s.	v.r.[a]	*P*[b]
pc × offspring sex × bs × replicate	170	2520·839	14·829	–	
E(null)	1	1123·641	1123·641	446·91	***
E(null) × bs	3	37·002	12·334	4·58	* (**)
E(null) × bs × replicate	14	37·674	2·691	–	
E(null) × replicate × pc	45	110·575	2·457	–	
Residual	11928	11144·486	0·934		
Total	12161	14974·217	1·231		

[a] Variance ratios are calculated using the mean square for *E*(null) × bs × replicate as the denominator.
[b] The asterisks in parentheses indicate a comparison with the pooled m.s. for *E*(null) × bs × replicate and *E*(null) × replicate × pc.
bs, bottleneck size treatment.
This notation also applies to Tables 5 and 6.

Table 5. *Analysis of covariance from the GLM of offspring values with the midparental values adjusted by* V_{MP} *as the explanatory variate* (*all treatments*)

Source of variation	d.f.	s.s.	m.s.	v.r.	*P*
pc × offspring sex × bs × replicate	170	2520·839	14·829	–	
E(null)	1	986·728	986·728	172·72	***
E(null) × bs	3	63·123	21·041	3·68	*
E(null) × bs × replicate	14	79·988	5·713	–	
E(null) × replicate × pc	45	149·446	3·321	–	
Residual	11928	11174·093	0·937		
Total	12161	14974·217	1·231		

Notation as for Table 4.

Table 6. *Analysis of covariance from the GLM of offspring values with* E(*molecular*) *as the explanatory variate* (*all treatments*)

Source of variation	d.f.	s.s.	m.s.	v.r.	*P*
pc × offspring sex × bs × replicate	170	2520·839	14·829	–	
E(molecular)	1	1136·689	1136·689	428·78	***
E(molecular) × bs	3	21·525	7·175	2·71	ns (*)
E(molecular) × bs × replicate	14	37·112	2·651	–	
E(null) × replicate × pc	45	113·116	2·514	–	
Residual	11928	11144·936	0·934	–	
Total	12161	14974·217	1·231		

Notation as for Table 4.

using either *E*(demographic) or *E*(molecular), gave essentially the same result. The analysis of h^2 for *E*(molecular), which predicts the greatest change, is shown in Table 6. The outcome is ambiguous: the change in h^2 does not differ significantly from the expected change when the treatment effect is compared with the $E \times \text{bs} \times \text{replicate}$ mean square, but it does differ significantly ($P < 0{\cdot}05$) when compared with the pooled mean square.

It was found that the behaviour of h^2 and V_A with respect to bottleneck size did not differ significantly among principal components. This was also apparent from a visual comparison of the separate graphs for each principal component. Therefore, a useful summary is provided by the average h^2 and V_A for each bottleneck size across PC1–6 (Fig. 5). Standard errors were calculated from the variation among replicates, rather than principal components. The V_A values were ln-transformed as their distributions were skewed and the treatment effects were expected to be multiplicative. The values expected from the neutral additive model, using the molecular estimates of F_t

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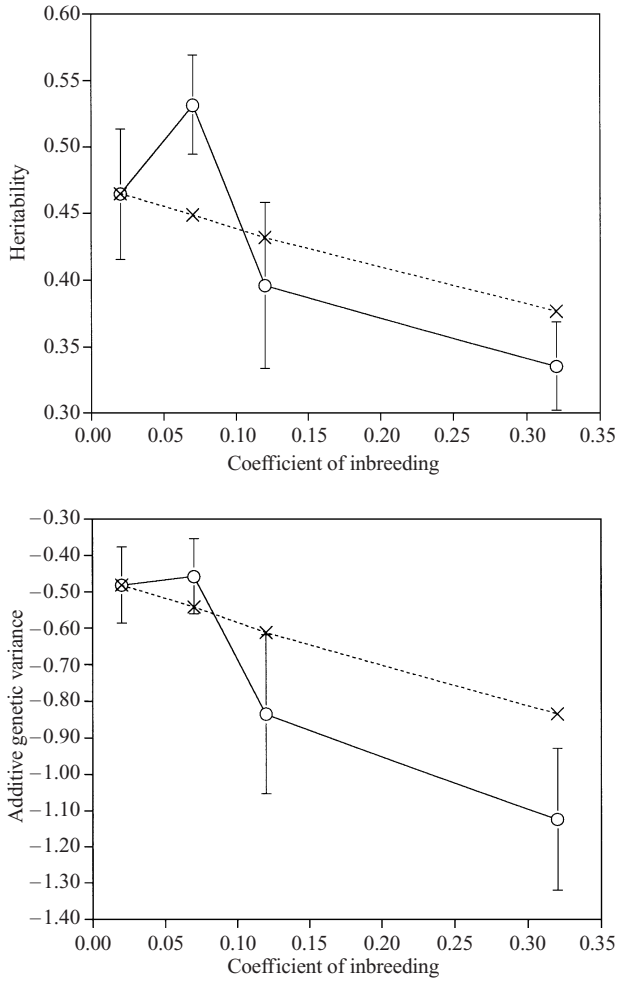

Fig. 5. Observed (continuous line) and expected (dashed line) heritability and ln-transformed additive genetic variance for each bottleneck size (coefficient of inbreeding) averaged across PC1–6. Standard errors describe the variation among replicates.

(relative to control F_t), are superimposed on both graphs.

Fig. 5 illustrates the general pattern of treatment effects on h^2 shown by the individual principal components and characters, confirming that the smaller bottlenecks caused heritability to decline; the biggest reduction occurred for the one pair bottleneck, followed by the three pair bottleneck. The average h^2 for the 10 pair bottleneck is marginally higher, but not significantly different, from the control average h^2. The expected h^2 is within 2 standard errors of the observed h^2 for all bottleneck sizes. Similarly, Fig. 5 shows large reductions in V_A for the one pair and three pair bottlenecks, but no change in V_A for 10 pairs relative to control lines. As was indicated by the analysis of variance, there is some evidence that average losses in one pair lines, and to a lesser extent in three pair lines, were greater than predicted by the neutral additive model. The close concordance of the 10 pair and control V_A values suggests that h^2 differences between these two treatments (see Fig. 5) were due to environmental variation.

Table 7 indicates that inbreeding did not alter V_E, and, not surprisingly, that principal components have different V_E. There was also no evidence for V_E differing among replicate lines.

4. Discussion

Our experiment indicates that bottlenecks of one pair and three pairs of individuals result in large and significant losses in h^2 and V_A for wing size and pattern (as analysed using principal components) in *Bicyclus anynana*. Furthermore, conservative interpretation of our statistical analysis indicates that these changes are in quantitative agreement with the neutral additive model of quantitative genetic variation.

There is some evidence to suggest that the losses were greater than expected under the neutral additive model, but it has not been possible to demonstrate this unequivocally (Table 6). Greater than expected losses in h^2 and V_A imply undetected inbreeding. For the observed and expected values of h^2 and V_A to coincide, the average level of inbreeding of the one pair and three pairs lines (relative to the control level of inbreeding) would need to be close to 0·48 and 0·32, respectively. Analysis of molecular data (Saccheri *et al.*, 1999) revealed that the experimental lines had indeed undergone more inbreeding than was previously thought (Table 1), probably as a result of increased variance in reproductive viability and background selection (hitch-hiking). This additional inbreeding, which is incorporated in the analysis with the *E*(molecular) expectation, may explain much of the loss in additive genetic variation (Table 6). Given

Table 7. *ANOVA of environmental variance*

Source of variation	d.f.	s.s.	m.s.	v.r.	*P*
pc	5	2·285	0·457	9·33	***
bs	3	0·198	0·066	1·35	ns
pc × bs	15	0·320	0·021	0·44	ns
Replicate	14	0·497	0·036	0·73	ns
Residual	70	3·428	0·049		
Total	107	6·729	0·063		

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the low level of replication imposed by the system, the remaining discrepancy could plausibly be accounted for by the variance of the realization of the drift process and error on parameter estimates (Lynch, 1988). Alternative mechanistic explanations based on extreme distributions of allelic effects (conceivably leading to greater than expected losses in V_A) are less plausible, both on theoretical grounds (Maynard Smith, 1998, p. 120) and because they are incompatible with the evidence for several genes, each of small phenotypic effect, controlling eyespot size (Wijngaarden, 2000).

A strength of our analysis is the use of principal components, rather than raw characters, because it allows a rigorous examination of the treatment effects for the measured set of characters as a whole and overcomes the problem of correlations faced by analyses of raw characters (e.g. Bryant *et al*., 1986). In this respect, an important result is that the effect of bottleneck size on both h^2 and V_A is consistent for the first six principal components. For several of these specific traits (viz. eyespot elements) strong evidence of a common genetic and developmental determination system (Brakefield *et al*., 1996) further justifies the use of principal components. Moreover, from an evolutionary perspective, the most likely target of selection in nature is the wing pattern as a whole (Brakefield, 2000), which is described more effectively by the principal components than the individual characters.

The additive behaviour of the wing pattern and size traits in response to inbreeding is consistent with the lack of any detectable inbreeding depression for these traits. The implication is that there is relatively little (directional) dominance variance for these traits which, as mentioned in Section 1, has been found to be the most common source of non-additive to additive variance conversion. The apparent lack of directional dominance for the wing traits in *B. anynana* conforms to the now well supported hypothesis (Kearsey & Kojima, 1967; Crnokrak & Roff, 1995; DeRose & Roff, 1999) predicting low directional dominance for traits under weak stabilizing selection, such as are most morphological traits. We have also detected very large directional dominance for a major component of fitness, egg hatching rate, in the same experimental lines (Saccheri *et al*., 1996), as expected for fitness-related traits subject to strong directional selection (DeRose & Roff, 1999). The rapid and large response to natural selection for increased egg hatching rate in some of the one pair lines (Saccheri *et al*., 1996), coupled with the observed increase in phenotypic variance, implies substantial increases in V_A for egg hatching rate, as a result of inbreeding. Thus, our results, which demonstrate losses of V_A for morphological traits and a probable increase in V_A for a major component of fitness, provide strong support for the view that increases in V_A following inbreeding will most often be due to large dominance variance in traits which are closely related to fitness.

Existing empirical evidence does not rule out the possibility that epistasis may also contribute substantially to increases in V_A of some characters following inbreeding. Because of the difficulties inherent in accurately estimating even the simplest form of epistatic variance (additive × additive) few studies of inbreeding effects, including our own, have attempted to measure it. In one inbreeding experiment where additive dominance (V_D), epistatic (V_{AA}), and environmental variances were measured (Bryant & Meffert, 1996) the results were somewhat equivocal, but did show a positive association between increases in V_A and V_{AA} in the base population, among four morphological characters. The results of Cheverud *et al*. (1999), while clearly demonstrating a potentially major role for epistatic to additive variance conversion, were obtained in a highly derived population (F2 intercross of inbred high and low selected lines), leading us to question the relevance of this result for natural populations. In future, the application of molecular quantitative genetics (QTLs) to describe the genetic architecture of traits in natural populations should provide greater insights into the role of non-additive variance components for adaptation in the presence of drift.

Even when non-additive variance is negligible, V_A may increase above control levels in a small fraction of cases, simply because of the variance about the expectation of the drift process, which is likely to be particularly large for polygenic traits. This has been demonstrated by the highly replicated *Drosophila* experiment of Whitlock & Fowler (1999). The same study found highly variable but significant overall increases in V_E for four of six wing area and angle measurements, paralleling the results of Bryant & Meffert (1996). Such increases in V_E are traditionally attributed to lowered developmental homeostasis, though the genetic basis and developmental mechanism for this hypothesis are unclear. We were unable to detect any significant effect of inbreeding on V_E for the *B. anynana* traits under investigation. This may be because of substantial differences in developmental canalization (Gibson & Wagner, 2000) or less stressful environmental conditions (e.g. less crowding).

Another factor that should be taken into consideration is that in natural (as opposed to laboratory) environments, founding colonists or population crash survivors are unlikely to be a random set from the base population. With the exception of the statistically problematic experiment of Lints & Bourgois (1984) who measured an increase in V_A for sternopleural bristle number following an extended bottleneck induced by high temperature, this possibility has not been explored by any of the empirical studies aiming

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to assess the consequences of bottlenecks for V_A. If, for example, the more heterozygous genomes are favoured, for which there is increasing evidence (Keller *et al*., 1994; Saccheri *et al*., 1998), V_A for the selected traits themselves or for those traits in linkage disequilibrium with the selected regions may increase, or decrease less than predicted by the neutral additive model.

There now exists an accumulated body of work indicating that bottlenecks are likely to cause losses in V_A of most characters most of the time, but increases in others. The evolutionary consequences of bottlenecks are therefore less predictable than implied by the neutral additive model. An important consideration is the relative fitness contribution of characters which gain V_A and those that lose V_A. Future studies aiming to make a general assessment of the effect of bottlenecks on quantitative characters should choose characters carefully, including a range of contrasting genetic architectures (i.e. varying relative magnitudes of additive, dominance and epistatic variances) and presumed selective regimes (major determinants of fitness versus weakly selected traits). The present study has also emphasized the need to obtain accurate estimates of the level of inbreeding when interpreting the effects of bottlenecks on quantitative genetic variation, and suggests that cryptic selection (Saccheri *et al*., 1999) can accelerate the loss of V_A.

Estimates of heritabilities, phenotypic variances and means are available on request from I.J.S.

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