Genet. Res., Camb. (2005), **85**, *pp.* 47–55. *With* 1 *figure.* © 2005 Cambridge University Press
doi:10.1017/S0016672305007342 Printed in the United Kingdom

Comparative losses of quantitative and molecular genetic variation in finite populations of *Drosophila melanogaster*

DEAN M. GILLIGAN[1]‡, DAVID A. BRISCOE[1] AND RICHARD FRANKHAM[1,2,3,4]*
[1] *Key Centre for Biodiversity and Bioresources, Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia*
[2] *School of Tropical Biology, James Cook University, Townsville, Qld 4811, Australia*
[3] *Program for Evolutionary Dynamics and Department of Organismic Biology, Harvard University, One Brattle Square level 6, Cambridge, MA 02138, USA*
[4] *Australian Museum, 6 College Street, Sydney, NSW 2010, Australia*

(*Received 21 April 2004 and in revised form 26 October and 5 December 2004*)

Summary

Quantitative genetic variation, the main determinant of the ability to evolve, is expected to be lost in small populations, but there are limited data on the effect, and controversy as to whether it is similar to that for near neutral molecular variation. Genetic variation for abdominal and sternopleural bristle numbers and allozyme heterozygosity were estimated in 23 populations of *Drosophila melanogaster* maintained at effective population sizes of 25, 50, 100, 250 or 500 for 50 generations, as well as in 19 highly inbred populations and the wild outbred base population. Highly significant negative regressions of proportion of initial genetic variation retained on inbreeding due to finite population size were observed for both quantitative characters ($b = -0{\cdot}67 \pm 0{\cdot}14$ and $-0{\cdot}58 \pm 0{\cdot}11$) and allozyme heterozygosity ($b = -0{\cdot}79 \pm 0{\cdot}10$), and the regression coefficients did not differ significantly. Thus, quantitative genetic variation is being lost at a similar rate to molecular genetic variation. However, genetic variation for all traits was lost at rates significantly slower than predicted by neutral theory, most likely due to associative overdominance. Positive, but relatively low correlations were found among the different measures of genetic variation, but their low magnitudes were attributed to large sampling errors, rather than differences in the underlying processes of loss.

1. Introduction

Genetic variation is the raw material required for populations to adapt to environmental change (Frankham *et al.*, 2002). Heterozygosity for neutral markers is predicted to be lost through drift in random mating populations as follows (Frankham *et al.*, 2002):

$$H_t/H_0 = [1 - 1/(2N_e)]^t = 1 - F \quad (1)$$

where H_t is heterozygosity at generation t, H_0 the original heterozygosity, N_e the effective population size and F the inbreeding coefficient. Loss of genetic variation for allozymes in *Drosophila* populations is in reasonable accord with predictions from this equation (Montgomery *et al.*, 2000).

However, adaptation to changing climate, diseases, parasites, etc., is primarily achieved by evolution of quantitative characters (Frankham *et al.*, 2002). Additive quantitative genetic variation (V_A) for a two-allele model is a function of heterozygosity for quantitative trait loci (QTL) as follows (Falconer & Mackay, 1996):

$$V_A = \sum 2pq[a + d(q - p)]^2 \quad (2)$$

where $2pq$ is the expected frequency of heterozygotes at a locus, a is half the difference between the means of the two homozygotes, d is the dominance deviation, p and q are allele frequencies and the effects are summed across all loci affecting the trait. With a completely additive model ($d = 0$), neutral additive genetic variance is also lost at the rate of $1/(2N_e)$

* Corresponding author. e-mail: rfrankha@els.mq.edu.au
‡ Current address: NSW Department of Primary Industries Science and Research Division – Aquatic Ecosystems, Narrandera Fisheries Centre, PO Box 182, Narrandera, NSW, Australia, 2700.

per generation as a result of slow drift (Falconer & Mackay, 1996), resulting in an equation similar to equation (1):

$$V_A(t)/V_{A(0)} = 1 - F \quad (3)$$

where $V_{A(t)}$ is the additive genetic variation at generation t and $V_{A(0)}$ the initial additive variation.

Despite these expectations, there is controversy concerning the strength of relationships between quantitative genetic variation and effective population size or inbreeding (Houle, 1989) and whether it differs from the relationship for molecular genetic variation (see below). Some studies have reported increases in quantitative genetic variation in populations subjected to bottlenecks (Bryant *et al*., 1986; López-Fanjul & Villaverde, 1989; Fernández *et al*., 1995; Wade *et al*., 1996). These have typically involved fitness or other characters showing substantial amounts of non-additive genetic variation and inbreeding depression where non-linear relationships between quantitative genetic variation and F are expected (Robertson, 1952; Willis & Orr, 1993; Cheverud *et al*., 1999). Quantitative genetic variation is often not neutral or additive, especially that for reproductive fitness (Frankham *et al*., 2002). Most quantitative characters (apart from fitness) are subject to stabilizing selection, while fitness is subject to directional selection. Therefore, the strength of the relationship between genetic variation and N_e is likely to be weaker for genetic markers more closely associated with fitness (Frankham, 1996) than for molecular markers such as allozymes where selective forces are weak (Frankham *et al*., 2002).

This report concentrates on peripheral quantitative characters showing predominantly additive genetic variation subject to weak natural selection and exhibiting little or no inbreeding depression, where a linear decline in quantitative genetic variation with F is predicted. Even for such peripheral characters there is disagreement about the relationship. Lopez-Fanjul *et al*. (1989), Whitlock & Fowler (1999), Frankham (2000) and Saccheri *et al*. (2001) have reported results for *Drosophila* and butterflies that agree with neutral predictions. Further, responses to artificial selection are greater in large than small populations, as expected from neutral theory (see Eisen, 1975; Weber & Diggins, 1990; Wade *et al*., 1996). Conversely, Cheverud *et al*. (1999) reported no decline in additive genetic variation for adult body weight in 55 mouse populations with $F = 0{\cdot}39$. Further, van Oosterhout & Brakefield (1999) failed to find a significant relationship between quantitative genetic variation for wing pattern characters and inbreeding coefficients (F) in the butterfly *Bicyclus anynana*. To date, the evidence on the relationship between quantitative genetic variation and population size is based on studies with a limited range of inbreeding coefficients, often few generations of restricted population size and sometimes with limited replication. Lynch (1988) has emphasized the need for extensive replication and several generations of restricted population size in studies to compare theoretical and observed changes in genetic variation and pointed to the lack of large reliable data sets.

Correlations between molecular and quantitative genetic variation across populations are typically low, averaging 0·2, and they do not differ significantly from zero for life-history characters (Reed & Frankham, 2001). The low correlations could be due to different patterns of change in genetic variation for the molecular and quantitative characters, or could simply be a reflection of large sampling errors around estimates of genetic diversity, especially those for quantitative traits. The former explanation would lead to different regressions of proportions of initial genetic diversity retained against inbreeding coefficient for molecular and quantitative characters. Conversely, the latter hypothesis would lead to regression coefficients that would not differ significantly.

The aims of this experiment were to quantify the relationships between quantitative genetic variation for peripheral characters and inbreeding due to finite population sizes and to compare the strength of those relationships with that for allozyme heterozygosity. Genetic variation for abdominal and sternopleural bristle number and allozyme heterozygosity were estimated from replicated populations of *Drosophila melanogaster* maintained at N_e's ranging from 25 to 500 for 50 generations, from 19 highly inbred populations, and from their wild outbred base populations. Abdominal and sternopleural bristle numbers are peripheral characters showing predominantly additive genetic variation and exhibiting little or no inbreeding depression in wild populations (Latter & Robertson, 1962; Sheridan *et al*., 1968; Lynch & Walsh, 1998). This study has a combination of a wider range of inbreeding coefficients than any prior study and longer duration, as well as extensive replication.

2. Materials and methods

(i) *Stocks*

An outbred strain of *D. melanogaster* (T92) was founded from 272 wild-inseminated females caught at Tyrrells Winery, Pokolbin, in eastern NSW, Australia, in February 1992. New populations from Tyrrells were established in 1996 (273 inseminated females; T96) and 1997 (104 inseminated females; T97). The Tyrrells population has been found to be stable in allozyme frequencies from the 1970s to the 1990s (see Frankham & Loebel, 1992), in microsatellite frequencies from 1992 to 1996 (England,

https://doi.org/10.1017/S0016672305007342 Published online by Cambridge University Press

1997) and in fitness from 1992 to 1994 (Woodworth *et al*., 2002).

Outbred *Drosophila* populations were expanded to 1000 adult parents per generation by the second generation in captivity and maintained at that size in 20×270 ml bottles per generation (about 25 pairs of parents per bottle) on potato-sugar (PS) medium (Frankham *et al*., 1988). These populations have effective sizes of approximately 300, based on loss of allozyme variation (Gilligan, 2001). Outbred populations were maintained at 18 °C to increase the generation time to 4 weeks.

Twenty-three N_e populations were founded from the T92 wild outbred population after two generations in captivity and maintained as fully pedigreed, random mating populations with effective sizes of 25 (8 replicates), 50 (6), 100 (4), 250 (3) and 500 (2) for 50 generations using single pair matings and equalization of family sizes. Flies were maintained in 100 mm × 25 mm glass vials on PS medium at 25 °C. After 50 generations, these populations were maintained as large random mating populations with 1000 parents per generation (25 pairs in each of 20 bottles with mixing of bottles each generation) at 18 °C on PS medium for a further 39 generations before measurement of quantitative genetic variation, thereby allowing linkage disequilibrium to decay.

A series of 50 highly inbred lines (FS populations) were founded from T96, each from a single pair mating. After 20 generations of full-sib mating 19 FS populations remained, each having a pedigree inbreeding coefficient of 0·986. Quantitative genetic variation was measured in these populations after one generation as stocks.

(ii) *Measuring quantitative genetic variation*

To maximize precision, quantitative genetic variation (V_G) was estimated using a method devised by Clayton *et al*. (1957), as

$$V_G = \sigma^2_P - \sigma^2_d \quad (4)$$

where σ^2_P is the phenotypic variance for fourth plus fifth abdominal bristle number (or left plus right sternopleural scores) and σ^2_d is the variance of the difference between the two counts on the same individual. This method was chosen as heritabilities based on such data have much lower standard errors for the same number of individuals scored than those estimated using offspring–parent regressions or sib-correlations (Frankham *et al*., 1968). This measures the total genetic variation, plus a small component of between-fly common environmental effects. It has proven to be a reliable means for measuring changes in quantitative genetic variation and to be stable over long periods under controlled environmental conditions (see Reeve & Robertson, 1954; Clayton & Robertson, 1957; Latter, 1964; Frankham *et al*., 1968; Frankham & Nurthen, 1981; Frankham, 2000). In brief, this method is dependent on environmental correlations between repeat segments being close to zero, genetic correlations between repeat segments being nearly unity and differences between repeat bristle segments (fluctuating asymmetry) being unrelated to inbreeding.

For abdominal bristles, empirical estimates have shown that the genetic correlation between adjacent segments is ~ 1 (Reeve & Robertson, 1954; Sheridan *et al*., 1968; Coyne & Beecham, 1987), while the environmental correlation is known to be very low for abdominal bristles (Reeve & Robertson, 1954). For sternopleurals, Reeve (1960) found that only 2% of phenotypic variance was due to genetic variance for sternopleural asymmetry, while Monedero *et al*. (1997) failed to detect mutational variance for fluctuating asymmetry. Therefore, the genetic correlation between scores on the left and right sides of the fly is ~ 1. Reeve (1960) reported environmental correlations between scores on left and right sides of the fly for inbred lines that ranged from 0·02 to 0·28 with a mean of 0·056. As the inbred lines were unlikely to be completely homozygous (Rumball *et al*., 1994), the environmental correlation is likely to be ~ 0.

Fluctuating asymmetry has previously been shown to be unrelated to inbreeding coefficient for sternopleural bristles in our populations (Gilligan *et al*., 2000). The variance of the difference between the two abdominal bristle counts on the same individual was unrelated to inbreeding coefficient for the data described in this paper, based upon a weighted regression ($r^2 = 0{\cdot}000$, $F_{1,40} = 0{\cdot}01$, $P = 0{\cdot}93$).

The Clayton *et al*. (1957) method measures total genetic variation whilst the theory above refers to additive genetic variation. Abdominal and sternopleural bristle numbers exhibit primarily additive genetic variation (see above), so their behaviour is expected to approximate the predictions. The small amount of non-additive genetic variation they exhibit will lead the comparisons of rates of loss of molecular and quantitative genetic variation with F to be biased in the direction of more deviation from theoretical expectations for the quantitative characters. Similar rates of loss for molecular and quantitative characters will indicate that they are being affected by similar processes, but if they show different patterns then non-additive genetic variation cannot be exclude as an explanation. Non-additive genetic variation also contributes to more conventional estimates of 'additive' genetic variation from parent–offspring regressions and sib-correlations (Lynch & Walsh, 1998), especially for full-sib correlations, as used by Cheverud *et al*. (1999).

For each N_e and FS population and T92 at generation 87, four 270 ml bottles were set up, each bottle housing 25 pairs of parent flies maintained at 25 °C on PS medium, similar to methods used previously (e.g. Frankham, 2000). Parent flies were allowed short laying-periods to achieve consistent low densities in all bottles. On day 7, pupal counts were taken for each bottle and the bottle that differed most from all others in each population was discarded. Emergent females were collected from within a 12 hour period on day 10. Twenty randomly chosen females per bottle were scored for bristle number on the fourth and fifth abdominal sternites and the right and left sternopleural plates. Larger numbers of bottles were scored for T96 (14) and T97 (5). All bristle scoring was done by the senior author in early to mid-1997, apart from an earlier assessment on T92 at generation 4 in 1992 by RF for abdominal bristles only, scoring 10 females from each of 20 bottles.

(iii) *Measuring allozyme heterozygosity*

The FS populations were assayed by protein electrophoresis for seven polymorphic gene-enzyme systems: acid phosphatase (ACP), α-glycerophosphate dehydrogenase (α-GPDH), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGDH), phosphoglucomutase (PGM), as described by Borlase *et al.* (1993), and esterase (EST-6) as described by Richardson *et al.* (1986). Both individuals (single pair matings) of the founding pair and the twentieth generation of full-sib mating were assayed, representing typing of the complete inbred population in each generation. Allozyme heterozygosity was characterized as the mean of Hardy–Weinberg expected heterozygosities across loci. Three FS populations were omitted from allozyme analyses as samples were not available for generation 20. Analyses restricted to the same 38 populations with data for all characters did not alter the major conclusions.

Heterozygosity estimates for the N_e populations at generation 49 were described by Montgomery *et al.* (2000). For the purposes of this analysis, the N_e population data were combined with estimates from the FS populations.

(iv) *Data analyses*

Proportions of molecular or quantitative genetic variation retained for each replicate population of each treatment were regressed on population inbreeding coefficients. Based on the theoretical expectation from equations (1) and (3), the neutral expectations are for an intercept of 1, and a slope of -1.

Estimates of quantitative genetic variation from replicate bottles of each population were averaged and the mean used in the regression. To allow comparisons between the relationships of molecular and quantitative genetics with F, measures of quantitative variation were divided by those in the population that most closely reflected the base population and had the highest precision (T92 G4 for abdominals, and T96 for sternopleurals), so that they had a similar structure to H_t/H_0 for allozymes. Weighted regressions were used for abdominals and sternopleurals as the sample sizes were not constant for all populations. Weighting was by number of individuals per population. The regression coefficients for both abdominal and sternopleural bristles were compared with that for allozyme heterozygosity using t-tests.

The inbreeding coefficient was used to assess the impacts of finite population size on genetic variation following equations (1) and (3), as populations had different numbers of generations at small size. For the N_e and FS populations, the inbreeding coefficient was obtained from pedigrees (Falconer & Mackay, 1996; Montgomery *et al.*, 2000), with the additional inbreeding during mass random matings in stocks for quantitative genetic variation assessments being computed from the effective size (300) and the number of generations using equation (1). While loss of genetic variation is dependent upon drift effective population size, inbreeding and drift sizes are the same when population sizes are constant (Crow & Kimura, 1970), as was the case in our experiment until populations were put into stock, and for the subsequent period in stock.

As effective population sizes are lower for sex-linked than autosomal loci, the inbreeding coefficients were adjusted for the proportion of sex-linked loci. For allozymes, one of the seven loci (6PGDH) was sex-linked, so the inbreeding coefficient was adjusted upwards by estimating the long-term effective size from observed F in each replicate using equation (1), calculating $[1-1/(2N_e)]^{6/7}[1-1/(1{\cdot}5N_e)]^{1/7}$, and estimating the adjusted F values from equation (1). A proportion of the quantitative genetic variation for both abdominal and sternopleural bristle numbers is known to be due to sex-linked loci (Spickett & Thoday, 1966; Frankham, 1969, 1977). As approximately 20% of the *Drosophila* eukaryotic genome is X-linked, the inbreeding coefficients have been adjusted upwards based on this proportion, using the same procedure as above. The adjustments for sex-linked loci made no differences to the conclusions for the quantitative loci.

Bristle number data were checked for outliers, using the Boxplot facility in Minitab, and obvious outliers removed. Quantitative genetic variation estimates for both abdominal and sternopleural bristle numbers differed significantly from normality (Ryan-Joiner test in Minitab). Square root and logarithmic transformations on population means were evaluated

and the former proved the better, with transformed data not differing significantly from normality. Frankham (2000) previously used a square root transformation to normalize quantitative genetic variation estimates for abdominal bristle number. However, the slopes of relationships with F were substantially altered with the transformed data. Since we wish to compare the relationships between quantitative and molecular data and the theory is formulated on an untransformed scale, we present analyses both for the untransformed data and for the square root transformed data. The conclusions as to the significance of relationships are usually the same for the two analyses.

Tests were done to determine whether linear or quadratic terms, or a combination of both, best explained the relationships between proportion of initial genetic variation retained and F. Correlations among different estimates of genetic variation across populations were computed and tested using one-tailed tests. Statistical analyses were done using Minitab version 12.

3. Results

Genetic variation was highly significantly negatively related to F for abdominal and sternopleural bristle numbers, as well as for allozymes (Fig. 1; Table 1. Consequently, both molecular and quantitative genetic variation are related to effective population size. The regressions for the two quantitative characters were highly significant regardless of whether they were done with the untransformed measures or the square root transformed estimates of quantitative genetic variation. The regressions were also significant when the base populations were removed, and when the highly inbred populations were separately omitted (analyses not shown).

The regression coefficients of genetic variation on F for abdominal and sternopleural bristle numbers on the untransformed scale were not significantly different from that for allozyme heterozygosity, ($t=0{\cdot}70$, d.f. $=79$, $P=0{\cdot}49$ and $t=1{\cdot}41$, d.f. $=79$, $P=0{\cdot}16$, respectively), and the regression coefficients did not differ between the two quantitative characters ($t=0{\cdot}51$, d.f. $=82$, $P=0{\cdot}61$).

The slopes of the regressions for abdominal and sternopleural bristle numbers and allozymes all differed significantly from the neutral expectation of $b=-1$ (Table 1). Intercepts did not differ significantly from the neutral expectation of $a=1$ for abdominals and allozymes, but did for sternopleurals on the untransformed scale. Inbreeding explained 37% and 43% of the genetic variation in abdominal and sternopleural bristle numbers (untransformed scale) and 62% of the variation in heterozygosity for allozymes.

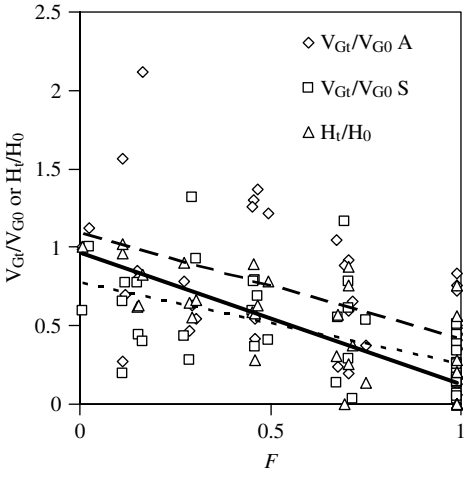

Fig. 1. Relationships between proportions of initial genetic variation retained and inbreeding coefficient for two quantitative characters (abdominal and sternopleural bristle numbers) and allozyme heterozygosity in 40–43 populations maintained with effective population sizes ranging from 2·6 to 500 for 20–50 generations. Long-dashed line, quantitative genetic variation for abdominal bristle number V_{Gt}/V_{G0} A; short-dashed line, quantitative genetic variation for sternopleural bristle number V_{Gt}/V_{G0} S; continuous line, allozyme heterozygosity (H_t/H_0).

A separate set of analyses was done on the highly inbred populations, compared with the base populations. The simple expectation was that the inbreds with $F=0{\cdot}986$ would retain only 0·014 of their initial genetic variation. For allozymes, the inbreds retained $0{\cdot}125\pm0{\cdot}058$ of their initial variation, while for abdominals and sternopleurals the corresponding figures were $0{\cdot}407\pm0{\cdot}059$ and $0{\cdot}214\pm0{\cdot}033$, based on comparisons with T92 generation 4 and T96, using untransformed data. Wilcoxon signed rank tests on the ratios showed that sternopleurals did not differ from allozymes ($P=0{\cdot}12$), while abdominals did ($P=0{\cdot}033$).

Tests to determine whether the best fitting relationships were linear or quadratic for the quantitative characters were done using regressions on F and F^2 and comparing the proportions of variation retained (r^2). Further, multiple regressions were done using F and F^2 terms entered in both orders. The F^2 relationships fitted marginally better than linear ones (r^2 of 0·375 versus 0·371 for abdominals and 0·439 versus 0·426 for sternopleurals), but there was no significant improvement in fit when fitting F after F^2, or *vice versa*. A similar test for allozyme heterozygosity indicated that the best fit was a linear relationship with F, but the difference in r^2 between that and the F^2 relationship was small (0·617 versus 0·601).

Correlations between the three measures of genetic variation were all positive, but relatively low (abdominals–sternopleurals: $r=0{\cdot}25$, $P=0{\cdot}059$; abdominals–allozyme heterozygosity: $r=0{\cdot}38$,

https://doi.org/10.1017/S0016672305007342 Published online by Cambridge University Press

Table 1. *Relationships between proportions of initial genetic variation retained and inbreeding coefficient for two quantitative characters (abdominal and sternopleural bristle numbers) and allozyme heterozygosity in 40–43 populations maintained with effective population sizes ranging from 2·6 to 500 for 20–50 generations. The neutral expectation is a linear regression with an intercept of 1 and a slope of −1*

Character	Intercept (±SE)	$P_{a=1}$	Regression coefficient (±SE)	$P_{b<0}$	$P_{b=-1}$	r^2
Allozyme heterozygosity	0·913±0·072	0·24	−0·786±0·100	<0·001	0·040	0·62
Quantitative genetic variation						
Untransformed linear						
Abdominals	1·088±0·089	0·33	−0·668±0·136	<0·001	0·019	0·37
Sternopleurals	0·827±0·072	0·02	−0·581±0·105	<0·001	<0·001	0·43
Square root transformed linear						
Abdominals	1·039±0·053	0·46	−0·419±0·082	<0·001	<0·001	0·39
Sternopleurals	0·913±0·054	0·12	−0·447±0·079	<0·001	<0·001	0·44
Untransformed quadratic						
Abdominals	1·004±0·075	0·96	−0·629±0·127	<0·001	NA	0·38
Sternopleurals	0·744±0·059	<0·001	−0·534±0·094	<0·001	NA	0·44

P values are tests for intercept $a=1$ (two-tailed test), regression coefficient $b<0$ (one-tailed) and for regression coefficient $b=-1$ (two-tailed).
NA, not applicable.

$P=0·01$; and sternopleurals–allozyme heterozygosity: $r=0·22$, $P=0·10$).

4. Discussion

Quantitative genetic variation for two peripheral characters and molecular variation were all negatively related to population inbreeding coefficient, and the relationships for the quantitative characters were not significantly different from that for molecular variation. Thus, quantitative genetic variation for peripheral characters is lost in small populations.

Previous work on peripheral quantitative characters has predominantly shown reductions in quantitative genetic variation with *F* and shown a significant inverse relationship between response to artificial selection and population size (see Section 1). Further, Briscoe *et al.* (1992) showed that quantitative genetic variation for sternopleural bristle number declined with generations in captivity for *Drosophila* population cages. However, these significant relationships contrast with the results of Cheverud *et al.* (1999) and van Oosterhout & Brakefield (1999).

The latter study found no significant relationships between h^2 and *F* for populations of butterflies with N_e from 6 to 24. van Oosterhout & Brakefield's inability to identify a relationship was probably a result of the narrow range and small N_e used, short duration, low replication and consequent low statistical power. Further, the observed proportional differences in heritabilities between the most and least inbred treatments were very close to those expected, even if non-significant. In contrast, we had high statistical power in our experiment with over 40 populations, N_e from 2·6 (full-sibs) to 500 and durations of 20 or 50 generations, so that our populations spanned almost the full range of inbreeding coefficients. Further, in small populations, random linkage disequilibrium can cause an increase in variance among replicates and obscure the real relationship (Bulmer, 1980, p. 231; Frankham, 2000). In this experiment, the N_e populations had time in stock to reduce linkage disequilibrium, but the FS populations did not. The FS populations did not seem to behave abnormally, as there were significant relationships whether they were included or excluded from the analyses and their exclusion slightly weakened the strength of the relationships (analyses not shown). Linkage disequilibrium will not be a factor in those inbred populations that became fixed, but may have contributed to variation among replicates in those that retained polymorphisms at some QTL loci.

The difference between our results and those of Cheverud *et al.* (1999) are harder to account for as they had 55 replicate populations with $F=0·39$, so statistical power is less likely to be the explanation. Nor should dominance be involved, as they reported no dominance variation for body weight in their population. Our study had a much wider range of inbreeding levels and a quadratic function fitted marginally better than a linear one for both quantitative characters. A comparison of our treatment with the nearest comparable *F* to that in Cheverud *et al.*'s experiment ($N_e=50$ with an average *F* of 0·41 and 6 replicates) revealed that the proportion of the initial quantitative genetic variance retained was 1·02 for abdominals and 0·60 for sternopleurals, one being similar to theirs and the other different. From the

https://doi.org/10.1017/S0016672305007342 Published online by Cambridge University Press

best-fitting quadratic relationships for our experiments, the predicted values of proportions of quantitative genetic variation retained were 0·90 for abdominals and 0·66 for sternopleurals. Thus, the differences between our results and those of Cheverud *et al.* (1999) are more apparent than real, especially for abdominal bristle number. The major difference seems to be that we had a much larger range of F values in our experiment. Cheverud *et al.* (1999) attributed the deviation from expectation in their experiment to epistatic genetic variation.

The relationships between genetic variation and population size may be weaker for quantitative characters related to fitness (Frankham, 1996) where balancing natural selection and the impacts of non-additive genetic variation are expected to be greater (see Charlesworth & Hughes, 2000). Three data sets have shown reduced evolvability in populations with smaller population sizes (Frankham *et al.*, 1999; Frankham *et al.*, 2002, p. 235; Reed *et al.*, 2003), but they do not resolve this issue. As prior small population size reduced fitness and selection differentials, any loss of quantitative genetic variation could not be distinguished from effects of reduced selection differentials. Clearly, data on a broader array of species and especially for reproductive fitness characters and for populations in nature would be desirable to resolve the issue raised by Bryant *et al.* (1986) and others.

The current results at first seem to be in conflict with the view that correlations between molecular and quantitative genetic variation are low (see Reed & Frankham, 2001). However, the correlations were also low in our experiment, and they were also low between quantitative genetic variation for the two quantitative traits. As the regressions of genetic variation on F were similar for allozymes and the two quantitative traits, the weak correlations point to low precision of estimates of quantitative variation as a major reason. From our results, there is no reason to doubt that quantitative genetic variation for traits peripheral to fitness is lost in small populations in a similar manner to near neutral molecular variation.

As allozymes have lower mutation rates than quantitative characters they regenerate genetic variation at a slower rate (Frankham *et al.*, 2002). While this will reduce the relationship between genetic variation and population size for quantitative characters compared with allozymes, it should have modest impacts over the time spans of this experiment. Times to regenerate allozyme variation are 10^5 to 10^7 generations and a few hundred to a few thousand generations for quantitative genetic variation, if populations regain large sizes (Lande & Barrowclough, 1987).

The rate of loss of both quantitative and molecular genetic variation was slower than predicted by the neutral model. Further, the highly inbred populations retained more genetic variation for both allozymes and quantitative variation than expected from neutral theory. The mean proportions of genetic variation retained were 0·41, 0·21 and 0·125 for abdominals, sternopleurals and allozymes, compared with the neutral expectation of 0·014. These data are comparable to the results of Rumball *et al.* (1994) who found that allozyme heterozygosity declined at a 20% slower rate than predicted in inbred lines from an earlier sample from the same *Drosophila* population. van Oosterhout & Brakefield (1999) also reported maintenance of high heritabilities in their inbred populations of butterflies, as did Tantawy & Reeve (1956) for their inbred populations of *Drosophila*. Several studies across a range of species have reported retention of allozyme variation in highly inbred populations (see Rumball *et al.*, 1994). The patterns in our experiments, where intercepts for abdominals and allozyme heterozygosity did not differ from unity, but deviations from neutrality built up with time, point to associative overdominance as the primary cause of the deviations (see Rumball *et al.*, 1994; Montgomery *et al.*, 2000). Simulations by Latter (1998) have shown that associative overdominance is expected in such populations of *Drosophila*. Similar patterns across different characters strengthen this conclusion.

In conclusion, rates of loss of molecular and quantitative genetic variation were similar in finite populations, but slower than predicted by neutral theory. Low correlations between molecular and quantitative genetic variation for peripheral characters are due predominantly to high sampling variation.

We thank M. Montgomery, L. Woodworth and E. Lowe for developing the N_e populations, K. Lees for developing the FS populations, R. Nurthen for assistance with allozyme electrophoresis, and J. Barker, W. Hill, B. Latter, D. Reed and two anonymous reviewers for comments on the manuscript. R.F. thanks V. Loeschcke for hosting him at the University of Aarhus during the revision of this work. This research was supported by Australian Research Council and Macquarie University research grants. Publication number 384 of the Key Centre for Biodiversity and Bioresources.

References

Borlase, S. C., Loebel, D. A., Frankham, R., Nurthen, R. K., Briscoe, D. A. & Daggard, G. E. (1993). Modeling problems in conservation genetics using captive *Drosophila* populations: consequences of equalization of family sizes. *Conservation Biology* **7**, 122–131.

Briscoe, D. A., Malpica, J. M., Robertson, A., Smith, G. J., Frankham, R., Banks, R. G. & Barker, J. S. F. (1992). Rapid loss of genetic variation in large captive populations of *Drosophila* flies: implications for the genetic management of captive populations. *Conservation Biology* **6**, 416–425.

Bryant, E. H., McCommas, S. A. & Combs, L. M. (1986). The effect of an experimental bottleneck upon

quantitative genetic variation in the housefly. *Genetics* **114**, 1191–1211.
Bulmer, M. G. (1980). *The Mathematical Theory of Quantitative Genetics*. Oxford: Clarendon Press.
Charlesworth, B. & Hughes, K. A. (2000). The maintenance of genetic variation in life-history traits. In *Evolutionary Genetics from Molecules to Morphology* (ed. R. S. Singh & C. Krimbas), pp. 369–392. Cambridge: Cambridge University Press.
Cheverud, J. M., Vaughn, T. T., Pletcher, L. S., King-Ellison, K., Bailiff, J., Adams, E., Erickson, C. & Bonislawski, A. (1999). Epistasis and the evolution of additive genetic variation in populations that pass through a bottleneck. *Evolution* **53**, 1009–1018.
Clayton, G. A. & Robertson, A. (1957). An experimental check on quantitative genetical theory. II. Long-term effects of selection. *Journal of Genetics* **55**, 152–170.
Clayton, G. A., Knight, G. R., Morris, J. A. & Robertson, A. (1957). An experimental check on quantitative genetical theory. III. Correlated response. *Journal of Genetics* **55**, 171–180.
Coyne, J. A. & Beecham, E. (1987). Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics* **117**, 727–737.
Crow, J. F. & Kimura, M. (1970). *An Introduction to Population Genetics Theory*. New York: Harper and Row.
Eisen, E. J. (1975). Population size and selection intensity effects on long-term selection response in mice. *Genetics* **79**, 305–323.
England, P. R. (1997). Conservation genetics of population bottlenecks. PhD thesis, Macquarie University, Sydney.
Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*. Harlow, UK: Longman.
Fernández, A., Toro, M. A. & Lopez-Fanjul, C. (1995). The effect of inbreeding on the redistribution of genetic variability of fecundity and viability in *Tribolium castaneum*. *Heredity* **75**, 376–381.
Frankham, R. (1969). Genetic analysis of two abdominal bristle selection lines. *Australian Journal of Biological Sciences* **22**, 1485–1495.
Frankham, R. (1977). The nature of quantitative genetic variation in *Drosophila*. III. Mechanism of dosage compensation for sex-linked abdominal bristle polygenes. *Genetics* **85**, 185–191.
Frankham, R. (1980). The founder effect and response to artificial selection in *Drosophila*. In *Selection Experiments in Laboratory and Domestic Animals* (ed. A. Robertson), pp. 87–90. Farnham, UK: Royal Commonwealth Agricultural Bureaux.
Frankham, R. (1996). Relationship of genetic variation to population size in wildlife. *Conservation Biology* **10**, 1500–1508.
Frankham, R. (2000). Modeling problems in conservation genetics using laboratory animals. In *Quantitative Methods for Conservation Biology* (ed. S. Ferson & M. Burgman), pp. 259–273. New York: Springer.
Frankham, R. & Loebel, D. A. (1992). Modeling problems in conservation genetics using captive *Drosophila* populations: rapid genetic adaptation to captivity. *Zoo Biology* **11**, 333–342.
Frankham, R. & Nurthen, R. K. (1981). Forging links between population and quantitative genetics. *Theoretical and Applied Genetics* **59**, 251–263.
Frankham, R., Jones, L. P. & Barker, J. S. F. (1968). The effects of population size and selection intensity in selection for a quantitative character in *Drosophila*. III. Analyses of the lines. *Genetical Research* **12**, 267–283.
Frankham, R., Yoo, B. H. & Sheldon, B. L. (1988). Reproductive fitness and artificial selection in animal breeding: culling on fitness prevents a decline in reproductive fitness in lines of *Drosophila melanogaster* selected for increased inebriation time. *Theoretical and Applied Genetics* **76**, 909–914.
Frankham, R., Lees, K., Montgomery, M. E., England, P. R., Lowe, E. & Briscoe, D. A. (1999). Do population size bottlenecks reduce evolutionary potential? *Animal Conservation* **2**, 255–260.
Frankham, R., Ballou, J. D. & Briscoe, D. A. (2002). *Introduction to Conservation Genetics*. Cambridge, UK: Cambridge University Press.
Gilligan, D. M. (2001). Conservation genetics and long term survival: testing models using *Drosophila*. PhD thesis, Macquarie University, Sydney.
Gilligan, D. M., Woodworth, L. M., Montgomery, M. E., Nurthen, R. K., Briscoe, D. A. & Frankham, R. (2000). Can fluctuating asymmetry be used to detect inbreeding and loss of genetic diversity in endangered populations? *Animal Conservation* **3**, 97–104.
Houle, D. (1989). The maintenance of polygenic variation in finite populations. *Evolution*, **43**, 1767–1780.
Lande, R. & Barrowclough, G. F. (1987). Effective population size, genetic variation, and their use in population management. In *Viable Populations for Conservation* (ed. M. E. Soulé), pp. 87–123. Cambridge: Cambridge University Press.
Latter, B. D. H. (1964). Selection for a threshold character in *Drosophila*. I. An analysis of phenotypic variance on the underlying scale. *Genetical Research* **5**, 198–210.
Latter, B. D. H. (1998). Mutant alleles of small effect are primarily responsible for the loss of fitness with slow inbreeding in *Drosophila melanogaster*. *Genetics* **148**, 1143–1158.
Latter, B. D. H. & Robertson, A. (1962). The effects of inbreeding and artificial selection on reproductive fitness. *Genetical Research* **3**, 110–138.
López-Fanjul, C. & Villaverde, A. (1989). Inbreeding increases genetic variance for viability in *Drosophila melanogaster*. *Evolution* **43**, 1800–1804.
López-Fanjul, C., Guerra, J. & Garcia, A. (1989). Changes in the distribution of the genetic variance of a quantitative trait in small populations of *Drosophila melanogaster*. *Genetics, Selection, Evolution* **21**, 159–168.
Lynch, M. (1988). Design and analysis of experiments on random genetic drift and inbreeding depression. *Genetics* **120**, 791–807.
Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer.
Monedero, J. L., Chavarrías, D. & López-Fanjul, C. (1997). The lack of mutational variance for fluctuating and directional asymmetry in *Drosophila melanogaster*. *Proceedings of the Royal Society of London, Series B* **264**, 233–237.
Montgomery, M. E., Woodworth, L. M., Nurthen, R. K., Gilligan, D. M., Briscoe, D. A. & Frankham, R. (2000). Relationships between population size and genetic diversity: comparisons of experimental results with theoretical predictions. *Conservation Genetics* **1**, 33–43.
Reed, D. H. & Frankham, R. (2001). How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* **55**, 1095–1103.
Reed, D. H., Lowe, E. H., Briscoe, D. A. & Frankham, R. (2003). Fitness and adaptation in a novel

environment: effect of inbreeding, prior environment, and lineage. *Evolution* **57**, 1822–1828.
Reeve, E. C. R. (1960). Some genetic tests on asymmetry of sternopleural chaeta number in *Drosophila*. *Genetical Research* **1**, 151–172.
Reeve, E. C. R. & Robertson, W. (1954). Studies in quantitative inheritance. VI. Sternite chaeta number in *Drosophila*: a metameric quantitative character. *Zeitschrift für Indukt Abstammungs- und Verebungslehre* **86**, 269–288.
Richardson, B. J., Baverstock, P. R. & Adams, M. (1986). *Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies*. Sydney, Australia: Academic Press.
Robertson, A. (1952). The effect of inbreeding on the variation due to recessive genes. *Genetics* **37**, 189–207.
Rumball, W., Franklin, I. R., Frankham, R. & Sheldon, B. L. (1994). Decline in heterozygosity under full-sib and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics* **136**, 1039–1049.
Saccheri, I. J., Nichols, R. A. & Brakefield, P. M. (2001). Effects of bottlenecks on quantitative genetic variation in the butterfly *Bicyclus anynana*. *Genetical Research* **77**, 167–181.
Sheridan, A. K., Frankham, R., Jones, L. P., Rathie, K. A. & Barker, J. S. F. (1968). Partitioning of variance and estimation of genetic parameters for various bristle characters of *Drosophila melanogaster*. *Theoretical and Applied Genetics* **38**, 179–187.
Spickett, S. G. & Thoday, J. M. (1966). Regular responses to selection. 3. Interaction between located polygenes. *Genetical Research* **7**, 96–121.
Tantawy, A. O. & Reeve, E. C. R. (1956). Studies in quantitative inheritance. IX. The effects of inbreeding at different rates in *Drosophila melanogaster*. *Zeitschrift für Indukt Abstamm -und Vererblehre* **87**, 648–667.
van Oosterhout, C. & Brakefield, P. M. (1999). Quantitative genetic variation in *Bicyclus anynana* metapopulations. *Netherlands Journal of Zoology* **49**, 67–80.
Wade, M. J., Shuster, S. M. & Stevens, L. (1996). Inbreeding: its effect on response to selection for pupal weight and the heritable variance in fitness in the flour beetle, *Tribolium castaneum*. *Evolution* **50**, 723–733.
Weber, K. E. & Diggins, L. T. (1990). Increased selection response in larger populations. II. Selection for ethanol vapor resistance in *Drosophila melanogaster*, at two population sizes. *Genetics* **125**, 585–597.
Whitlock, M. C. & Fowler, K. (1999). The change in genetic and environmental variance with inbreeding in *Drosophila melanogaster*. *Genetics* **152**, 345–353.
Willis, J. H. & Orr, H. A. (1993). Increased heritable variation following population bottlenecks: the role of dominance. *Evolution* **47**, 949–957.
Woodworth, L. M., Montgomery, M. E., Briscoe, D. A. & Frankham, R. (2002). Rapid genetic deterioration in captivity: causes and conservation implications. *Conservation Genetics* **3**, 277–288.