

Lack of genetic variation in developmental instability in zebra finch (*Taeniopygia guttata*) wing and tarsus

Stefan Van Dongen¹ and Wolfgang Forstmeier²

¹Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium and

²Max Planck Institute for Ornithology, Seewiesen, Germany

ABSTRACT

Question: How much additive genetic variance of developmental instability is maintained under a selection–mutation balance in a domesticated population of zebra finches (*Taeniopygia guttata*)?

Methods: We estimate the heritability and coefficient of additive genetic variance (CV_A) of wing and tarsus developmental instability from an animal model.

Results: No significant additive genetic variance was detected. Estimates of additive genetic variance, heritabilities, and CV_A were low, but, in contrast to the CV_A , moderate heritabilities could not be excluded due to highly skewed distributions.

Conclusions: Although domesticated for more than 100 years, reducing selection pressures, lowering genetic variation for developmental instability, and allowing the accumulation of *de novo* genetic variance, the CV_A of developmental instability is likely to be below 2–3% in this population.

Keywords: Bayesian analysis, developmental instability, evolvability, fluctuating asymmetry, genetic variation.

INTRODUCTION

Developmental instability (i.e. the sensitivity of a developing system to random noise) may reflect individual genetic quality and thus be the target of natural and/or sexual selection. This hypothesis originates from observed negative associations between fluctuating asymmetry (i.e. random deviations from perfect symmetry, which are assumed to reflect developmental instability) and fitness components. If directional selection favours more stable development, genetic variation for developmental instability may become depleted. The question is how much additive genetic variance is maintained in a population under selection–mutation balance. To be relevant in evolutionary ecology, developmental instability should not only relate to fitness – an aspect that has been debated for decades (e.g. Palmer, 1999; Thornhill *et al.*, 1999; Van Dongen, 2006) – but also show additive genetic variance. Although the study of the genetic basis of developmental instability dates back to the 1950s

Correspondence: Stefan Van Dongen, Evolutionary Ecology Group, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium. e-mail: stefan.vandongen@ua.ac.be

Consult the copyright statement on the inside front cover for non-commercial copying policies.

and 1960s (e.g. Mather, 1953; Reeve, 1960), it has received a lot of attention recently (e.g. Van Dongen and Talloen, 2007; Johnson *et al.*, 2008; Carter and Houle, 2011). A meta-analysis by Møller and Thornhill (1997a) initiated debates about the magnitude of the evolutionary potential of fluctuating asymmetry and developmental instability (e.g. Houle, 1997; Markow and Clarke, 1997; Palmer and Strobeck, 1997; Møller and Thornhill, 1997b), and inspired new analyses and more recent attempts to obtain average estimates (e.g. Whitlock, 1996; Van Dongen, 1998, 2000; Fuller and Houle, 2003). Yet, despite the large literature, a consensus about the genetic basis of fluctuating asymmetry and developmental instability eludes us. More recent reviews suggest very little evidence for substantial additive genetic variance of developmental instability (Fuller and Houle, 2003; Leamy and Klingenberg, 2005). Leamy and Klingenberg (2005) hypothesized that the genetic basis of fluctuating asymmetry is predominantly non-additive and character-specific, with occasional single-locus effects that affect fluctuating asymmetry *per se*. However, Fuller and Houle (2003) and Van Dongen (2007) highlighted that most available studies did not have sufficient sample sizes to estimate the evolvability of developmental instability reliably.

Some recent studies have found moderate heritabilities when combining data from different traits (e.g. Van Dongen and Talloen, 2007; Johnson *et al.*, 2008), thereby reducing the high sampling variation inherent to research on fluctuating asymmetry (Whitlock, 1996; Gangestad and Thornhill, 1999; Leamy and Klingenberg, 2005; and below). In addition, in a large-scale selection experiment, Carter and Houle (2011) demonstrated that developmental instability did respond to selection. Thus, a few studies appear to contradict previous reviews of the literature.

Further large-scale studies might help us to understand under which conditions developmental instability contains additive genetic variance (Leamy and Klingenberg, 2005; Van Dongen, 2007). The likelihood of detecting significant genetic variation may be increased in populations in which directional selection for more stable development (i.e. greater symmetry) has been relaxed. Organisms that have been domesticated for many generations should offer such an advantage. The zebra finch (*Taeniopygia guttata*) is a small Australian grassland songbird that has been domesticated over the past two centuries. Because it is easy to breed in captivity, it has become a widely used study organism, especially in behavioural research. Most zebra finches were brought to Europe between 1870 and 1890, with few new imports after the First World War. During its long period of captive breeding, sexual selection (e.g. through mate choice) and functional selection (e.g. on wing symmetry during flight) against asymmetry have probably been less intense compared with wild populations, thus providing the opportunity for the generation of new genetic variation by mutation. On the other hand, effective population sizes have been limited, unavoidably leading to a loss of some genetic variation (Forstmeier *et al.*, 2007). Nevertheless, the captive populations harbour a considerable amount of neutral genetic variation (Forstmeier *et al.*, 2007). In addition, high heritabilities of morphological traits have been reported for our study population (Bolund *et al.*, 2010b; Forstmeier *et al.*, 2010). Although we cannot exclude the possibility that genetic variation of developmental instability has been depleted by the relatively low effective population sizes, the zebra finch population may show a somewhat higher degree of additive genetic variance of developmental instability. The additional advantage of this domesticated species is that pedigrees over several generations are available and that genetic parameters can be estimated with relatively high accuracy using an animal model.

An important aspect of this research is that the observable fluctuating asymmetry in a single trait only weakly reflects the underlying process of developmental instability. Therefore, associations between fluctuating asymmetry and other covariates, as well as estimates of the heritability of fluctuating asymmetry, underestimate patterns in developmental

instability (Whitlock, 1996, 1998; Houle, 1997, 2000; Van Dongen, 1998; Gangestad and Thornhill, 1999). Thus obtaining estimates of genetic parameters of developmental instability requires very large sample sizes. Furthermore, even very low heritabilities in fluctuating asymmetry may translate into high heritabilities in developmental instability (e.g. Gangestad and Thornhill, 1999). An unbiased estimate of the heritability of developmental instability can be obtained by dividing the heritability of fluctuating asymmetry in a trait by its repeatability R (Whitlock, 1996). Because the value of R is theoretically bounded between 0 and 0.637 (Whitlock, 1996, and see below), but often is relatively small (e.g. Gangestad and Thornhill, 1999), this transformation can lead to large increases (of heritability of developmental instability compared with fluctuating asymmetry), but will also result in very wide confidence intervals. To complicate matters even more, inference at the level of the heritability of developmental instability must also take into account the sampling error associated with the estimation of R . Van Dongen (2007) showed that a Bayesian latent variable is capable of taking all sampling variation into account and provides unbiased estimates and correct credibility intervals (the latter are the Bayesian analogues of confidence intervals). Although the estimation of genetic parameters is known to be statistically demanding and to require relatively large sample sizes, recent simulations have shown that few, if any, studies to date had a sufficiently large sample size to estimate accurately the heritability of developmental instability (Fuller and Houle, 2003; Van Dongen, 2007; but see Carter and Houle, 2011, for a counterexample). Because very few model systems will yield sample sizes sufficient to obtain accurate estimates of heritabilities of developmental instability, information across different traits may be combined to increase sampling accuracy (e.g. Van Dongen and Talloen, 2007; Johnson *et al.*, 2008). Regardless, estimates of the evolutionary potential of developmental instability should be accompanied by measures of accuracy including all sources of sampling variation (Van Dongen, 2007). Also, Pélabon *et al.* (2004) argued that in studies of fluctuating asymmetry, the coefficient of additive variance (CV_A) of fluctuating asymmetry should be preferred over the heritability of fluctuating asymmetry because the former provides an unbiased estimate of the coefficient of additive variance of developmental instability. Because the coefficient of additive variance may better reflect evolvability in some cases (Houle, 1992), we also report it here.

In this paper, we provide estimates of the heritability and coefficient of additive variance of fluctuating asymmetry and developmental instability in tarsus and wing lengths of a large, captive-bred zebra finch population. We apply Monte Carlo Markov chain methods to provide distributions of the genetic parameters in order to describe their sampling variation as accurately as possible. A recently developed Bayesian model is extended to an animal model to make inference at the level of developmental instability, taking all sources of sampling variation into account (Van Dongen, 2007).

METHODS AND MATERIALS

Study population

Our zebra finches came from five successive generations (Parental through F4) of a domesticated laboratory population [described as population #18 in Forstmeier *et al.* (2007)]. The Parental and F1 generations were bred at the University of Sheffield, UK, and subsequently transported to the Max Planck Institute for Ornithology in Seewiesen, Germany, where the F2, F3, and F4 generations were bred and where all living birds were measured for fluctuating asymmetry.

Asymmetry measurements and estimation of developmental instability

From a total of 1480 birds without visible indications of wear or damage, or malformations of the legs and wings, we measured tarsus and/or wing length twice on both sides (sequence: left–right–left–right). Tarsus length (mean $\pm$ s.d. = 14.5 ± 0.51) was measured from the bent foot to the end of the tibiotarsus bone to the nearest 0.01 mm using calipers; wing length (mean $\pm$ s.d. = 59.1 ± 1.79) was measured to the nearest 0.5 mm using a wing ruler. We performed repeated measurements to separate real asymmetry from measurement error. However, measurements within a single session are not independent. To evaluate between-session measurement error, we repeated the same measurement procedure for a subset of 225 birds.

We applied a mixed regression model (Van Dongen *et al.*, 2009) to determine variation in real asymmetry and measurement error within and between sessions. We also tested for directional asymmetry using a *t*-test (Van Dongen *et al.*, 2009). Overall, the measurement error was of about the same magnitude as the real asymmetry. Between-session variation appeared larger than within-session variation in measurement error (Table 1). Directional asymmetry was observed, which may be due to handedness of the observer (Table 1).

Signed asymmetries were calculated as the best linear unbiased predictors from the mixed model, thereby correcting for directional asymmetry (Table 1). Signed asymmetry values were not highly correlated between the two traits ($r = 0.02$, $N = 1480$, $P = 0.34$), suggesting that they are not developmentally integrated and represent independent estimates of developmental instability. The absolute value of the signed asymmetries was not positively correlated with trait size (tarsus: $r = 0.011$, $N = 1480$, $P = 0.31$; wing: $r = -0.088$, $N = 1480$, $P < 0.01$), so we did not perform any size correction. The unsigned asymmetries of the two traits were not significantly positively correlated ($r_{FA} = 0.033$, $N = 1480$, $P = 0.20$), neither supporting the presence of an individual asymmetry parameter nor a joint effect of individual-specific developmental instability on both traits. This correlation in unsigned fluctuating asymmetry (*FA*), however, underestimates the latent correlation in developmental instability (r_{DI}). It is possible to transform r_{FA} into r_{DI} using the hypothetical repeatability R of both traits, i.e. $r_{DI} = r_{FA} / \sqrt{R_1 \times R_2}$ (Whitlock, 1996).

This value of R reflects the repeatability of the asymmetric development of a bilateral trait and is higher with increasing variation in developmental instability among individuals. The value of R can be estimated as:

$$\frac{2}{\pi} - \frac{\pi - 2}{\pi} \times \frac{1}{CV_{FA}^2},$$

Table 1. Estimated variances due to real asymmetry (FA) and within- and between-session measurement error

Trait	Real FA (%)	Measurement error		Directional asymmetry	R
		Between-session	Within-session		
Tarsus	0.018 (62%)	0.010	0.0015	$t_{1479} = -498$, $P < 0.0001$	0.18
Wing	0.153 (47%)	0.194	0.034	$t_{1479} = 8.1$, $P < 0.0001$	0.17

Note: The results of a *t*-test of directional asymmetry are provided in the penultimate column. The last column provides the hypothetical repeatability, R (Whitlock, 1998).

where CV_{EA} is the coefficient of variation of the unsigned fluctuating asymmetry. If there is no variation in developmental instability, $CV_{EA} = 0.76$ and $R = 0$. In the limit, the CV_{EA} will reach very high values and the upper limit of R is equal to $2/\pi = 0.637$ (Whitlock, 1996, 1998). In our population, the values of R were moderate (Table 1). The estimated correlation in developmental instability of both traits was 0.18 (i.e. $0.033 / \sqrt{0.17 \times 0.19}$), indicating that averaging information across the two traits could be biologically meaningful to reduce sampling error, although we cannot support this statistically. The consequence of this possible lack of correlation is that the heritabilities of fluctuating asymmetry based on the average fluctuating asymmetry will not be higher (and closer to the heritability of developmental instability) than single trait estimates (Johnson *et al.*, 2008).

Estimation of genetic parameters

We estimated the genetic parameters from animal models fitted using the software R (cran.r-project.org/; version 2.10.0) – not the study R (i.e. repeatability). We used the packages *pedigreemm* and *MCMCglmm*. We fitted a fully Bayesian model using *OPENBUGS* (openbugs.info/w/). In all analyses, we estimated three variance components: the additive genetic variance ($\sigma_a^2 = V_A$), the maternal effects ($\sigma_m^2 = V_M$), and the residual variance ($\sigma_e^2 = V_E$). First, we determined the significance of the additive genetic variance using permutations, i.e. we repeatedly randomly exchanged the order of the unsigned fluctuating asymmetry values across the pedigree. The P -values were the proportions of the V_A values obtained in the permutations that were larger than the observed value from the point estimate. Next, we obtained the additive genetic variance in *MCMCglmm* from the ‘animal effect’. To estimate the maternal effect, we added ‘mother identity’ (not linked to the pedigree) as a random effect. We added the degree of inbreeding (inbreeding coefficient based on a five-generation pedigree) as a fixed effect, because Bolund *et al.* (2010a) showed that it affects developmental instability in these traits in this population. The advantage of the MCMC approach is that realistic estimates of the sampling variation are obtained as the posterior distributions, without having to make unrealistic assumptions about the shape of those distributions. Posterior distributions were based on 250,000 iterations after a burn-in of 25,000 iterations and a thinning of 50 to avoid problems of autocorrelation.

Heritabilities ($h^2 = V_A / (V_A + V_M + V_E)$) were estimated for both traits separately and for average asymmetry. Because distributions of unsigned asymmetries are typically highly skewed, we also performed analyses on log-transformed asymmetries. Next, point estimates (posterior median) and upper and lower limits of the 95% highest posterior density (HPD) of the posteriors of h_{EA}^2 were transformed to h_{DI}^2 by dividing h_{EA}^2 by R for the single trait estimates (Whitlock, 1996, 1998). For the transformation of h_{EA}^2 based on the average asymmetry across the two traits, we followed Gangestad and Thornhill (1999). In addition, we provide coefficients of additive variance as a measure of relative evolvability following Houle (1992):

$$CV_A = \frac{\sqrt{V_A}}{\bar{X}} \times 100.$$

The advantage of this measure is that it may better reflect evolvability and can be used for comparative purposes (Houle, 1992). Furthermore, the CV_A of fluctuating asymmetry provides an unbiased estimate of the CV_A of developmental instability since it is not scaled by the

residual error V_E , because the latter is high for fluctuating asymmetry owing to its weak association with developmental instability (e.g. Whitlock, 1996; Pélabon *et al.*, 2004).

The heritability of fluctuating asymmetry underestimates the heritability of developmental instability (Whitlock, 1996, and above). Dividing h_{FA}^2 by R to estimate h_{DI}^2 as applied here, however, does not take the uncertainty in the estimation of R into account. Consequently, the amount of uncertainty in the estimation is underestimated, leading to overly narrow confidence limits (Van Dongen, 2007). In spite of the large sample size in this study, we show that the uncertainty in the estimation of R is still remarkably high (see below). Therefore, in a final analysis, we also applied a Bayesian model with developmental instability as a latent variable, to take all uncertainty into account (see also Van Dongen, 2007). In OPENBUGS, the animal model was parameterized as follows at the level of fluctuating asymmetry:

The unsigned asymmetries (FA) are considered modelled by a linear model (with a linear predictor abbreviated LP) with normal residual variation and common variance:

$$FA[i] \sim N(LP[i], \sigma_e^2).$$

The linear predictor consists of a fixed effects part modelling the association between fluctuating asymmetry and inbreeding, determined by the intercept and slope. In addition, an additive genetic effect a and maternal effect m were considered as random effects:

$$LP[i] = \beta_0 + \beta_1 \text{inbreeding} + a[i] + m[i].$$

The additive genetic component was determined as follows:

$$A[i] \sim N(\text{parent}.a[i], \sigma_a^2),$$

where the parental contribution was determined by:

$$\text{parent}.a[i] = 0.5 \times (a[SIRE] + a[DAM]).$$

The maternal effects were modelled as:

$$m[i] \sim N(0, \sigma_m^2).$$

To allow an analysis at the level of developmental instability, the model needs to be augmented by treating developmental instability as a latent variable, reflecting an individual-specific variance component, linked to the signed asymmetry as follows:

$$FA[i] \sim N(0, \sigma_{DI}^2[i]).$$

And the above model is then fitted for this latent variable ($\sigma_{DI}^2[i]$) as follows:

$$\sigma_{DI}^2[i] \sim N(LP[i], \sigma_e^2).$$

Bayesian models in OPENBUGS (openbugs.info/w/) were fitted for both traits separately. To obtain estimates averaged across both traits, we did not use a full multivariate model because it would have caused numerical problems. Instead, we first standardized the asymmetry values and then estimated a single joint additive genetic (i.e. $a[i]$) and maternal effect (i.e. $m[i]$) across both traits for each individual as follows:

$$FA_{\text{tarsus}}[i] \sim N(0, \sigma_{DI_tarsus}^2[i])$$

$$FA_{\text{wing}}[i] \sim N(0, \sigma_{DI_wing}^2[i])$$

$$\sigma_{DI_tarsus}^2[i] \sim N(LP_tarsus[i], \sigma_{e,tarsus}^2)$$

$$\sigma_{DI_wing}^2[i] \sim N(LP_wing[i], \sigma_{e,wing}^2)$$

$$LP_tarsus[i] = \beta_{0,tarsus} + \beta_{1,tarsus}inbreeding + a[i] + m[i]$$

$$LP_wing[i] = \beta_{0,wing} + \beta_{1,wing}inbreeding + a[i] + m[i].$$

All Bayesian analyses in OPENBUGS were based on five independent MCMCs. After a burn-in of 25,000 iterations, we ran 250,000 independent iterations with a thinning of 50 to avoid autocorrelation problems. Prior distributions were normal distributions with zero mean and variance of 10^6 for the linear components (β 's). For all variances, the prior distributions were defined at the level of the standard deviations as uniform distributions between 0 and 1.

RESULTS

Table 2 provides estimates of the different variance components and heritability estimates of fluctuating asymmetry (FA). There was no significant genetic variation in fluctuating asymmetry for tarsus ($P=0.52$), wing ($P=0.64$) or average asymmetry ($P=0.36$). Estimates

Table 2. Summary statistics of the posterior distributions of the variance components of the animal models

Trait		Variance components			Heritability ($V_A/(V_A + V_M + V_E)$)		
		V_A	V_M	V_E	mean (S.E.)	median	95% HDP
Tarsus	FA	0.000	0.000	0.009	0.002 (0.010)	0.000	0–0.015
	DI				0.004 (0.052)	0.000	0–0.082
	FA_log	0.000	0.003	0.151	0.006 (0.013)	0.000	0–0.047
	DI_log				0.035 (0.069)	0.002	0–0.249
	DI_BUGS	0.000	0.000	0.001	0.008 (0.027)	0.000	0–0.091
Wing	FA	0.000	0.005	0.009	0.001 (0.010)	0.000	0–0.015
	DI				0.010 (0.061)	0.000	0–0.092
	FA_log	0.000	0.012	0.386	0.005 (0.018)	0.000	0–0.042
	DI_log				0.027 (0.104)	0.000	0–0.224
	DI_BUGS	0.003	0.004	0.012	0.203 (0.170)	0.160	0–0.641
Average	FA	0.000	0.000	0.512	0.001 (0.008)	0.000	0–0.011
	DI				0.003 (0.030)	0.000	0–0.032
	FA_log	0.000	0.002	0.142	0.002 (0.009)	0.000	0–0.022
	DI_log				0.012 (0.054)	0.000	0–0.056
	DI_BUGS	0.000	0.001	0.005	0.112 (0.103)	0.073	0–0.311

Note: For the additive genetic variance (V_A), the maternal effects (V_M), and residual variance (V_E), point estimates are provided (posterior median). For heritability, the sampling variation is provided by the 95% highest posterior density (HPD). Results are presented for the unsigned asymmetry (FA) and developmental instability (DI) of both traits (tarsus and wing) and averaged across both traits. Estimates for the log-transformed data are also provided (FA_log and DI_log). Estimates for DI are calculated directly from FA estimates using the hypothetical repeatabilities from Table 1. DI_BUGS, in contrast, refers to modelled estimates that take into account the uncertainty in the estimation of repeatabilities.

of the heritability of fluctuating asymmetry were virtually zero, with the highest upper limit for wing asymmetry (Table 2). Posterior distributions of the heritabilities of asymmetry obtained in MCMCglmm showed high mass near zero (Fig. 1). The coefficient of additive variance was 0.3% (95% HPD: 0.00–7.9%) for tarsus FA, 0.1% (95% HPD: 0.00–2.3%) for wing FA, and 0.2% (95% HPD: 0.00–13.9%) for average FA. As for the heritability of fluctuating asymmetry, posterior distributions showed high mass close to zero and a relatively long but very flat right tail leading to somewhat higher upper limits (Table 2; Fig. 2). For the log-transformed asymmetries, estimates were somewhat higher for the heritabilities (FA_Log in Table 2). The fact that the heritability of fluctuating asymmetry for the average FA was not higher than the single trait estimates also supports the lack of an individual asymmetry parameter (IAP). In such cases, the estimates based on the average FA are not very meaningful because the fluctuating asymmetry of tarsus and wing do not reflect the same biological phenomenon.

By definition, estimates of heritability of developmental instability (DI) increase because they are obtained by using the hypothetical repeatabilities of Table 1 to transform the heritabilities in fluctuating asymmetry. Nevertheless, the posterior distribution showed a high concentration of mass near zero and a very long and flat right tail (Fig. 1). The upper limit of the 95% confidence interval of the heritability of developmental instability was smaller than 0.1 in each case (Table 2).

As outlined above, this conventional method of directly transforming heritabilities in fluctuating asymmetry into heritabilities in developmental instability using the hypothetical repeatability, R , does not take into account sampling error in the estimation of R . To

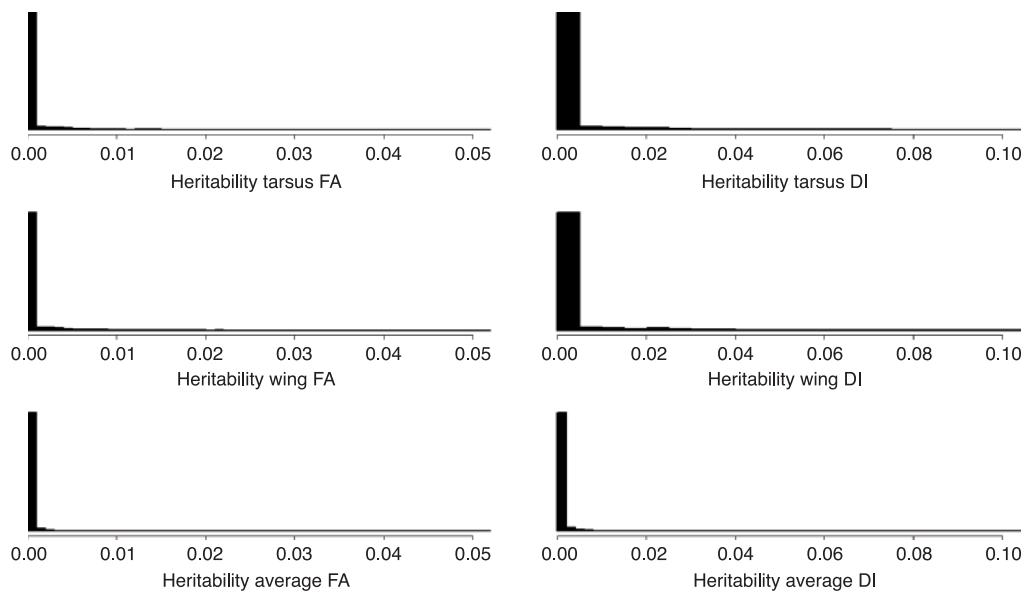


Fig. 1. Posterior densities of heritabilities of fluctuating asymmetry (FA) and developmental instability (DI) for zebra finch tarsus length, wing length, and asymmetry averaged across both traits. Estimates for DI were calculated based on the densities of the heritabilities of FA using formulae in Gangestad and Thornhill (1999). See text for details.

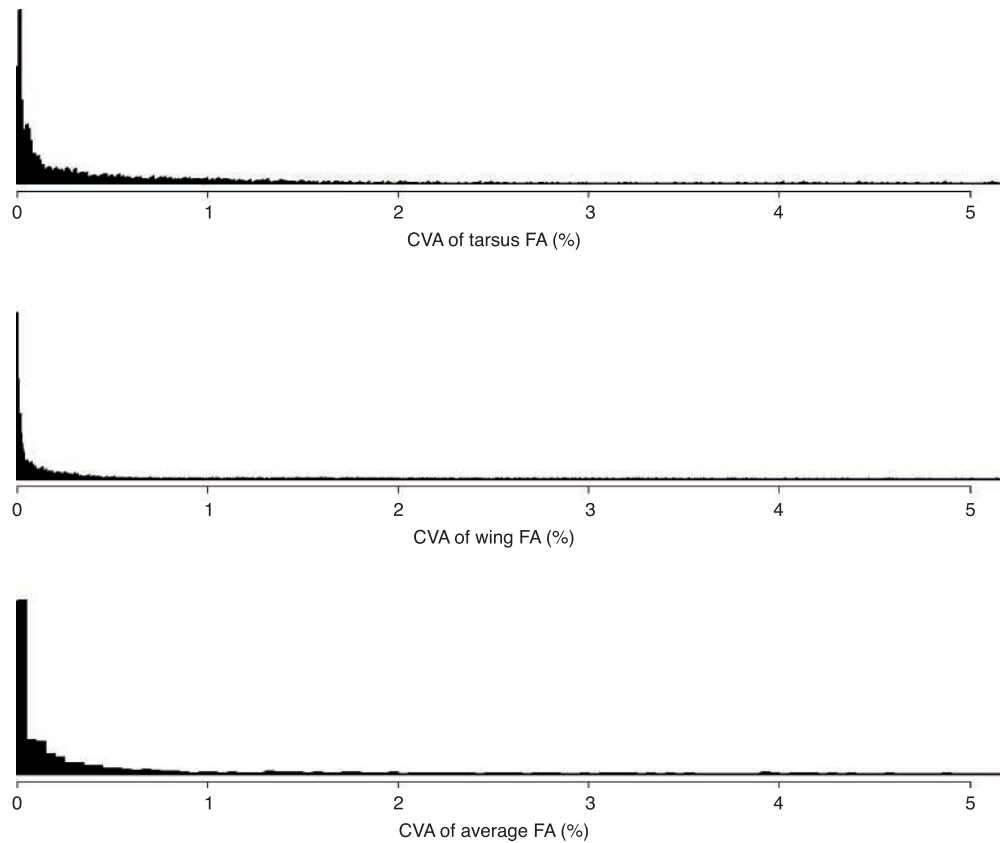


Fig. 2. Posterior distributions of the coefficients of additive variance (CV_A , expressed as a percentage) of tarsus, wing, and average fluctuating asymmetry (FA).

illustrate that this sampling error is substantial even with the large sample sizes in this study, we provide a bootstrap distribution of the hypothetical repeatabilities of the two traits in Fig. 3. Because the hypothetical repeatability is used in the denominator to obtain heritabilities of developmental instability, the lower tail of the distribution of R in particular is expected to contribute to the upper tail of the distribution of the heritability of developmental instability. We extended a recently developed Bayesian model (Van Dongen, 2007) – adapted to an animal model here – to obtain correct posterior distributions of the heritabilities of developmental instability. The posteriors indeed show even heavier right tails (Fig. 4), leading to substantially wider confidence intervals (DI_BUGS estimates in Table 2). Consequently, it is not possible to exclude moderate to even high heritabilities of developmental instability. However, as expected, the coefficients of additive variance of developmental instability were very similar to those for fluctuating asymmetry [0.1% (95% HPD: 0.00–2.2%) for tarsus DI, 0.7% (95% HPD: 0.00–2.7%) for wing DI, and 0.2% (95% HPD: 0.00–1.7%) for average DI], supporting the conclusion that developmental instability has low additive genetic variation (Fig. 4).

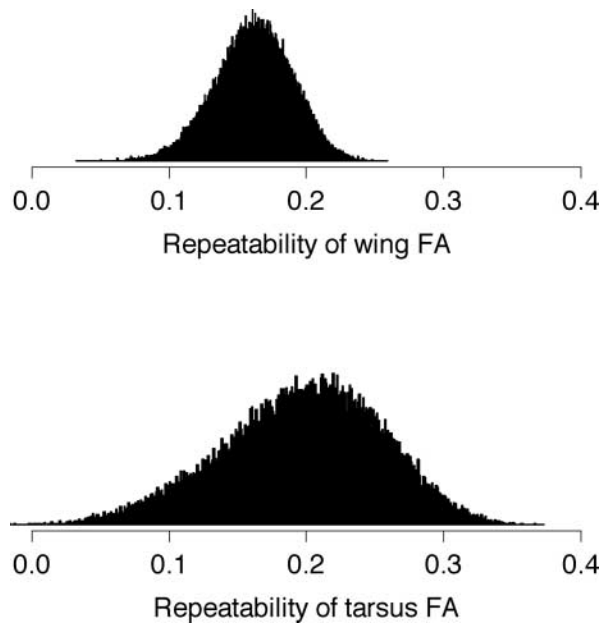


Fig. 3. Bootstrap distribution of the hypothetical repeatabilities of tarsus and wing asymmetry. The 95% confidence intervals ranged between 0.10 and 0.21 and between 0.08 and 0.30 for wing and tarsus fluctuating asymmetry, respectively.

DISCUSSION

Despite more than 60 years of research, the genetic basis of developmental instability remains poorly understood. Most recent reviews do appear to agree that there is little support for even moderate levels of additive genetic variance of developmental instability. The genetic basis of fluctuating asymmetry may be predominantly non-additive and character-specific, with occasional single-locus effects on fluctuating asymmetry *per se*. Yet some researchers have emphasized that more research is needed (Fuller and Houle, 2003; Leamy and Klingenberg, 2005). Indeed, some recent research suggests moderate heritabilities of developmental instability (Van Dongen and Talloen, 2007; Johnson *et al.*, 2008). A recent large-scale selection experiment with *Drosophila melanogaster* showed a response to selection for both increased and decreased fluctuating asymmetry (Carter and Houle, 2011). A significant obstacle is that the estimation of the heritability of developmental instability is prone to very high degrees of sampling variation, requiring unrealistically large sample sizes for most model species (e.g. Whitlock, 1996; Gangestad and Thornhill, 1999; Fuller and Houle, 2003; Van Dongen, 2007). This high uncertainty is due to the fact that the estimation of heritability involves a scaling using the total phenotypic variance. The total variance in fluctuating asymmetry, however, contains a relatively large amount of sampling variation because the fluctuating asymmetry of a single trait measures developmental instability very inaccurately (Whitlock, 1996). Consequently, heritabilities in fluctuating asymmetry are expected to be very low, and transformations into heritabilities in developmental instability do result in very wide confidence intervals (Fuller and Houle, 2003; Van Dongen 2007). The coefficient of additive genetic variance (CV_A) provides unbiased estimates (Pélabon *et al.*, 2004). Furthermore, CV_A 's may better reflect the

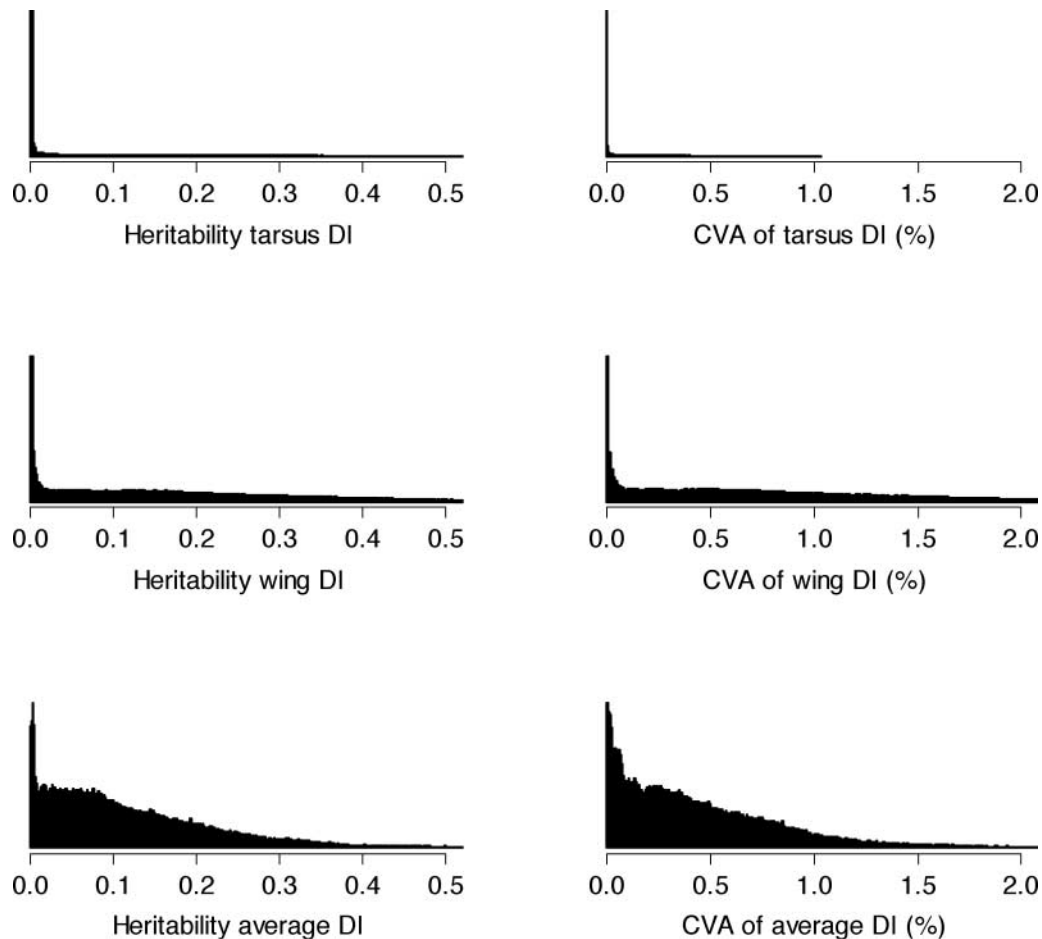


Fig. 4. Posterior densities of (left) heritabilities of developmental instability (DI) and (right) coefficients of additive variance (CV_A), for zebra finch tarsus length, wing length, and asymmetry averaged across both traits obtained in WINBUGS. Estimates were obtained using a latent variable approach adopted from Van Dongen (2007). See text for details.

evolvability of a trait and allow comparisons among traits (Houle, 1992). However, the CV_A does not have a simple biological interpretation in the same way heritability does. Combining information across traits should also reduce sampling variation considerably, provided the traits share variation in developmental instability (e.g. Gangestad and Thornhill, 1999). Although we studied two traits here, they did not provide evidence of a relatively high correlation in developmental instability. Thus, at least in the present case, combining information across traits did not increase accuracy significantly.

On average, heritabilities of fluctuating asymmetry were of about the same magnitude or somewhat lower compared with those reported in the literature (Fuller and Houle, 2003), and were not statistically different from zero. However, even with a sample of nearly 1500 birds, the uncertainty in the estimation remained substantial, especially for h^2_{DI} from the latent variable model. In spite of higher mass close to zero, heritabilities of developmental

instability close to 0.25 could not be excluded. The marked discrepancy between the conventional use of the hypothetical repeatability R to obtain heritabilities of developmental instability (Whitlock, 1996; Van Dongen, 1998) and the fully Bayesian approach (Van Dongen, 2007), emphasizes the need to take all uncertainty into account. We therefore conclude that our study provides relatively little information about the heritability of developmental instability. There is strong support for relatively low values (<0.1), but higher values cannot be ruled out with high confidence. As emphasized by Van Dongen (2007), estimating the heritability of developmental instability is statistically very demanding and cannot be performed with high accuracy. The scaling of the additive genetic variance by the total phenotypic variance when obtaining heritabilities, inherently results in high sampling variation, especially because the total phenotypic variance in fluctuating asymmetry overestimates that in developmental instability. Thus, not only because the coefficient of additive genetic variance may more closely reflect evolvability (Houle, 1992), but also because scaling is performed by the mean [not causing a downward bias (Pélabon *et al.*, 2004)], the coefficient of additive genetic variance appears more appropriate in studies of developmental instability.

Indeed, estimates of the coefficient of additive genetic variance allowed us to draw stronger conclusions in this study. Distributions of heritabilities were highly skewed to the right, medians were relatively low ($<0.5\%$), but upper limits suggest that the CV_A of developmental instability is likely to be below 3% in this population. As indicated above, the CV_A does not have a simple biological interpretation, but allows comparisons with other traits and studies. Although few studies on fluctuating asymmetry have reported CV_A values, the results from this zebra finch population suggest relatively low values compared with one previous empirical study. Polak and Starmer (2001) reported values of 8–13% for bristle number FA in *Drosophila falleni* and 30–45% for positional FA in these bristles. In addition, Gangestad and Thornhill (2003) anticipated high CV_A values based on estimates of the heritability of fluctuating asymmetry and variation in developmental instability. In contrast, several other studies reported similarly low CV_A values [e.g. 1.9% for mouse-mandible FA (Leamy, 1999); nearly 0% for *Dalechampia scandens* (Pélabon *et al.*, 2004); and negative values for earwigs (Tomkins and Simmons, 1999)]. Thus, although only a few studies have reported estimates of CV_A , and generalizations are premature, most evidence suggests relatively low evolvability. Perhaps not unexpectedly, the highest values of additive genetic variance of developmental instability are found in bristle number (Polak and Starmer, 2001), where it is likely that there is weaker selection against slight asymmetries because bristle number is not a trait related to locomotion.

In summary, the results of the present research are in general in line with recent reviews. Heritabilities were not statistically significant and very low for fluctuating asymmetry. However, transformation into heritabilities of developmental instability resulted in very wide confidence intervals, especially when all sources of uncertainty were incorporated in the model. Although there was stronger support for low heritabilities of developmental instability, moderate values of up to 0.25 could not be excluded. However, we conclude that estimating h^2_{DI} will require many thousands of individuals to be sampled. The CV_A , however, showed narrow confidence intervals and did support low evolvability with CV_A very likely to be below 2–3%. Although the interpretation of the CV_A is less straightforward compared with heritabilities, even this upper limit appears to correspond to relatively low evolvabilities in general (e.g. Houle, 1992; Hansen *et al.*, 2003).

In conclusion, we found no evidence for higher additive genetic variance in this population in spite of domestication and relaxed directional selection. Although the study

population harbours ample neutral (Forstmeier *et al.*, 2007) and additive genetic variance in several traits (Bolund *et al.*, 2010b; Forstmeier *et al.*, 2010), it is possible that repeated bottlenecks have depleted genetic variation for developmental instability more strongly, yet we do not see good arguments for why such selective depletion of V_A for developmental instability should have occurred. Nevertheless, future studies should include CV_A as a measure of evolvability of developmental instability because its estimates are much more accurate. Sample sizes of about 1500 individuals and the study of different traits appear to be the absolute minimum to be able to draw meaningful conclusions. And that minimum is only rarely achieved.

ACKNOWLEDGEMENTS

We thank X. Baecke, M. Jacobs, J. Elst, and H. Matheve for taking or assisting with morphological measurements; E. Bolund, K. Martin, and H. Schielzeth for help with breeding; S. Bauer, E. Bodendorfer, A. Grötsch, J. Hacker, M. Halser, J. Minshull, P. Neubauer, F. Preininger, M. Ruhdofer, A. Türk, and B. Wörle for animal care; and B. Kempenaers for good advice and support of all kinds. The study was supported by an Emmy-Noether Fellowship of the German Research Foundation (DFG; FO 340/1-2 and 1-3) to W.F., by the Max Planck Society, and by Research Program G.0025.07 of the Research Foundation – Flanders (FWO) to S.V.D.

REFERENCES

- Bolund, E., Martin, K., Kempenaers, B. and Forstmeier, W. 2010a. Inbreeding depression of sexually selected traits and attractiveness in the zebra finch. *Anim. Behav.*, **79**: 947–955.
- Bolund, E., Schielzeth, H. and Forstmeier, W. 2010b. No heightened condition dependence of zebra finch ornaments – a quantitative genetic approach. *J. Evol. Biol.*, **23**: 586–597.
- Carter, A. and Houle, D. 2011. Artificial selection reveals heritable variation for developmental instability. *Evolution*, **65**: 3558–3564.
- Forstmeier, W., Segelbacher, G., Mueller, J.C. and Kempenaers, B. 2007. Genetic variation and differentiation in captive and wild zebra finches (*Taeniopygia guttata*). *Mol. Ecol.*, **16**: 4039–4050.
- Forstmeier, W., Mueller, C.M. and Kempenaers, B. 2010. A polymorphism in the estrogen receptor gene explains covariance between digit ratio and mating behaviour. *Proc. R. Soc. Lond. B*, **277**: 3353–3361.
- Fuller, R.C. and Houle, D. 2003. Inheritance of developmental instability. In *Developmental Instability: Causes and Consequences* (M. Polak, ed.), pp. 157–181. Oxford: Oxford University Press.
- Gangestad, S.W. and Thornhill, R. 1999. Individual differences in developmental precision and fluctuating asymmetry: a model and its implications. *J. Evol. Biol.*, **12**: 402–416.
- Gangestad, S.W. and Thornhill, R. 2003. Fluctuating asymmetry, developmental instability, and fitness: toward model-based interpretation. In *Developmental Instability: Causes and Consequences* (M. Polak, ed.), pp. 62–80. Oxford: Oxford University Press.
- Hansen, T.F., Pélabon, C., Armbruster, W.S. and Carlson, M.L. 2003. Evolvability and genetic constraint in *Dalechampia* blossoms: I. Components of variance and measures of evolvability. *J. Evol. Biol.*, **16**: 754–766.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics*, **130**: 195–204.
- Houle, D. 1997. Comment on ‘A meta-analysis of the heritability of developmental stability’ by Møller & Thornhill. *J. Evol. Biol.*, **10**: 17–20.
- Houle, D. 2000. A simple model of the relationship between asymmetry and developmental stability. *J. Evol. Biol.*, **13**: 720–730.
- Johnson, W., Gangestad, S.W., Nancy, S.L. and Thomas, B.J. 2008. Heritability of fluctuating asymmetry in a human twin sample: the effect of trait aggregation. *Am. J. Human Biol.*, **20**: 651–658.

- Leamy, L. 1999. Heritability of directional and fluctuating asymmetry for mandibular characters in random-bred mice. *J. Evol. Biol.*, **12**: 146–155.
- Leamy, L.J. and Klingenberg, C.P. 2005. The genetics and evolution of fluctuating asymmetry. *Annu. Rev. Ecol. Syst.*, **36**: 1–21.
- Markow, T.A. and Clarke, G.M. 1997. Meta-analysis of the heritability of developmental stability: a giant step backward. *J. Evol. Biol.*, **10**: 31–37.
- Mather, K. 1953. Genetic control of stability in development. *Heredity*, **7**: 297–336.
- Møller, A.P. and Thornhill, R. 1997a. A meta-analysis of the heritability of developmental stability. *J. Evol. Biol.*, **10**: 1–16.
- Møller, A.P. and Thornhill, R. 1997b. Developmental instability is heritable. *J. Evol. Biol.*, **10**: 69–76.
- Palmer, A.R. 1999. Detecting publication bias in meta-analyses: a case study of fluctuating asymmetry and sexual selection. *Am. Nat.*, **154**: 220–233.
- Palmer, A.R. and Strobeck, C. 1997. Fluctuating asymmetry and developmental stability: heritability of observable variation vs. heritability of inferred cause. *J. Evol. Biol.*, **10**: 39–49.
- Pélabon, C., Hansen, T.F., Carlson, M.L. and Armbruster, W.S. 2004. Variational and genetic properties of developmental stability in *Dalechampia scandens*. *Evolution*, **58**: 504–514.
- Polak, M. and Starmer, W.T. 2001. The quantitative genetics of fluctuating asymmetry. *Evolution*, **55**: 498–511.
- Reeve, E.C.R. 1960. Some genetic tests on asymmetry of sternopleural chaeta number in *Drosophila*. *Genet. Res.*, **1**: 151–172.
- Thornhill, R., Møller, A.P. and Gangestad, S.W. 1999. The biological importance of fluctuating asymmetry and sexual selection: a reply to Palmer. *Am. Nat.*, **154**: 234–241.
- Tomkins, J.L. and Simmons, L.W. 1999. Heritability of size but not symmetry in a sexually selected trait chosen by female earwigs. *Heredity*, **82**: 151–157.
- Van Dongen, S. 1998. How repeatable is the estimation of fluctuating asymmetry? *Proc. R. Soc. Lond. B*, **265**: 1423–1427.
- Van Dongen, S. 2000. The heritability of fluctuating asymmetry: a Bayesian hierarchical model. *Ann. Zool. Fenn.*, **37**: 15–24.
- Van Dongen, S. 2006. Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. *J. Evol. Biol.*, **19**: 1727–1743.
- Van Dongen, S. 2007. What do we know about the heritability of developmental instability? Answers from a Bayesian model. *Evolution*, **61**: 1033–1042.
- Van Dongen, S. and Talloen, W. 2007. Phenotypic and genetic variations and correlations in multitrait developmental instability: a multivariate Bayesian model applied to Speckled Wood butterfly (*Pararge aegeria*) wing measurements. *Genet. Res.*, **89**: 155–163.
- Van Dongen, S., Wijnaendts, L.C.D., Ten Broek, C.M.A. and Galis, F. 2009. Fluctuating asymmetry does not consistently reflect severe developmental disorders in human fetuses. *Evolution*, **63**: 1832–1844.
- Whitlock, M. 1996. The heritability of fluctuating asymmetry and the genetic control of developmental stability. *Proc. R. Soc. Lond. B*, **263**: 849–853.
- Whitlock, M. 1998. The repeatability of fluctuating asymmetry: a revision and extension. *Proc. R. Soc. Lond. B*, **265**: 1429–1431.