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OPINION

The use (or misuse) of microsatellite allelic distances in the context of inbreeding and conservation genetics

BENGT HANSSON

Department of Animal Ecology, Ecology Building, Lund University, S-223 62 Lund, Sweden

Abstract

In line with inbreeding theory, genetic diversity at a set of molecular markers may explain variation in fitness-associated traits in partially inbred populations, and such associations will appear as 'genotype–fitness correlations'. An individual genetic diversity index specifically used for microsatellites is 'mean d^2', i.e. the mean squared distance between alleles. The original hypothesis for mean d^2–fitness correlations assumes that mean d^2 captures fitness effects at both ends of the inbreeding–outbreeding spectrum. This hypothesis received strong criticism from work showing that even a plain diversity estimate such as multi-locus heterozygosity (MLH) outperforms mean d^2 as a predictor of the inbreeding coefficient and fitness in most realistic situations. Despite this critique, the mean d^2-approach is still used frequently in ecological and evolutionary research, producing results suggesting that mean d^2 sometimes provides a stronger prediction of fitness than does MLH. In light of the critique, such results are unexpected, but potential explanations for them may exist (at least hypothetically), including scenarios based on close linkage and recent admixture. Nevertheless, a major caveat is that it is very difficult to predict a priori if mean d^2 will improve the genotype–fitness correlation, which in turn makes objective interpretations difficult. Mean d^2–fitness associations are potentially interesting, but the fact that we cannot easily understand them is problematic and should be thoroughly addressed in each study. Therefore, instead of hastily reached interpretations of mean d^2–fitness correlations, conclusions need support from complementary analyses, e.g. verifying admixture of genetically structured populations.

Keywords: admixture, fitness, heterozygosity, linkage disequilibrium, mean d^2, microsatellite, step-wise mutations

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Inbreeding depression and the original hypothesis for mean d^2–fitness correlations

It is well-established that inbreeding depresses survival and reproduction of individuals in most outcrossing species (reviewed in Keller & Waller 2002). Moreover, research on a few bottlenecked and isolated populations has shown, and confirmed worst-case predictions, that inbreeding poses a real threat to the long-term persistence of populations and can contribute towards driving populations to extinction (Saccheri *et al.* 1998; Westemeier *et al.* 1998; Madsen *et al.* 1999). At the same time, other severely bottlenecked and highly inbred populations seem unaffected by inbreeding and have managed to grow rapidly in numbers despite their depauperate genetic variability (Bonnell & Selander 1974; Komdeur 1994; Groombridge *et al.* 2000). These population-specific differences in how inbreeding strikes make it difficult to predict to what extent inbreeding, habitat fragmentation and population declines affect natural populations. Therefore, further

Correspondence: B. Hansson, Fax: +46462224719,
E-mail: bengt.hansson@zooekol.lu.se

studies quantifying inbreeding depression in wild unmanipulated species and populations are needed in conservation and evolutionary biology.

To study the effects of inbreeding, data on relatedness and inbreeding coefficients are necessary, and in some situations this can be achieved by constructing pedigrees. However, pedigree-building is problematic in many field populations, as several generations have to be monitored, individuals of unknown relatedness may immigrate, and extra-pair fertilizations make paternity assessment difficult. The molecular revolution of the 1980s offered an alternative approach, namely to estimate individual inbreeding coefficients by scoring the degree of heterozygosity at a set of molecular markers, and in this way separate inbred homozygous individuals from outbred heterozygous ones. In line with inbreeding theory, it was further proposed that positive correlations between the proportion of heterozygous markers of individuals, i.e. 'multi-locus heterozygosity' (MLH), and variation in fitness-associated traits would indicate selection against individuals with low genome-wide heterozygosity (reviewed in David 1998; Hansson & Westerberg 2002). An initial prerequisite for such associations is that there is some variation in the inbreeding coefficient in the population, i.e. that the population is partially inbred or in 'identity disequilibrium' (cf. David 1998; Hansson & Westerberg 2002; Balloux *et al.* 2004; Slate *et al.* 2004).

Coulson *et al.* (1998) suggested a novel approach to evaluate the fitness consequences of inbreeding and outbreeding from microsatellite genotype data. Their approach was based on the idea that microsatellites mutate according to the step-wise mutation model (SMM; Ohta & Kimura 1973; Levinson & Gutman 1987; Valdes *et al.* 1993; Schlötterer 2000): a slippage mutation process that either increases or decreases the length of the original microsatellite allele by one or a few repeat units. Under the SMM, microsatellite alleles of similar length are more likely to be related by descent than alleles of different length, and there will be an inherent 'temporal memory' in the allelic distance data that can be incorporated into the estimate of the inbreeding coefficient. Coulson *et al.* (1998) defined an individual microsatellite diversity index, called 'mean d^2', as the mean squared distance between microsatellite alleles within an individual:

$$\text{mean } d^2 = \sum_{i=1}^{n} \frac{(i_a - i_b)^2}{n},$$

where i_a and i_b are the length in repeat units of allele a and b at locus i, and n is the number of typed loci. The authors hypothesized that inbred individuals have low mean d^2, whereas a high mean d^2 indicates outbred individuals (Coulson *et al.* 1998, 1999; Pemberton *et al.* 1999). Furthermore, it was suggested that mean d^2 enables separation of individuals into those descending from short- and long-distance dispersing immigrants, respectively, and therefore that it will provide a better resolution of the inbreeding coefficient deep in the pedigree, and better capture admixture events, than MLH (Coulson *et al.* 1998, 1999; Pemberton *et al.* 1999). Data from microsatellites mutating according to the SMM had previously been used in a conceptually similar way for describing the coalescence time between populations (Goldstein *et al.* 1995; Slatkin 1995), but Coulson *et al.* (1998) were first to suggest that d^2 could be useful for estimating the genome-wide heterozygosity and inbreeding coefficient of individuals within populations.

Estimating the inbreeding coefficient with molecular markers in the wild poses a great challenge since many natural populations exhibit weak relatedness structures with moderate variation in the inbreeding coefficient. Random segregation of alleles induces substantial sampling variance in marker-based heterozygosity at a specific inbreeding level, especially when few markers are screened, which will often lead to poor correlations between the marker-based estimate and the true inbreeding coefficient in such populations (Balloux *et al.* 2004; Slate *et al.* 2004). Thus, modifications of the plain MLH-estimate in order to improve the fit to the inbreeding coefficient and strengthen the genotype–fitness correlation are welcomed. Indeed, other modifications of MLH than mean d^2 have been proposed, e.g. 'internal relatedness' (Amos *et al.* 2001) and 'heterozygosity by loci' (Aparicio *et al.* 2006), but yet none of them has attracted the same attention as the mean d^2-approach.

In line with predictions from the mean d^2-approach, it was detected that red deer (*Cervus elaphus*) and harbour seals (*Phoca vitulina*) which were heavier at birth and managed to survive the initial period of life had higher mean d^2 than individuals that were lean and had higher rate of mortality (Coltman *et al.* 1998; Coulson *et al.* 1998). It was suggested that the most likely source of allele-length variation in these populations was dispersal between diverged populations, i.e. admixture, and that individuals with high mean d^2 were fitter because they were heterozygous at many loci throughout the genome (Coltman *et al.* 1998; Coulson *et al.* 1998; Pemberton *et al.* 1999). Further analyses in the red deer population revealed sex-dependent associations between first-year overwintering survival and mean d^2 (Coulson *et al.* 1999). As predicted by inbreeding depression, female red deer caves with high mean d^2 survived better that those with low mean d^2. However, the direction was opposite in males, suggesting that male calves suffered from outbreeding depression due to admixture events in the population (Coulson *et al.*

1999). The hypothesis of using the mean d^2-approach to detecting fitness effects at both ends of the inbreeding–outbreeding continuum was accepted by many researchers and mean d^2 has now been used and found to correlate with fitness-associated trait in several populations (see e.g. Coltman & Slate 2003 and this study).

Criticism of the mean d^2-approach

However, other researchers were expressing their doubts over using mean d^2 as a proxy for inbreeding history (Hedrick *et al.* 2001; Tsitrone *et al.* 2001; reviewed in Goudet & Keller 2002; cf. Slate & Pemberton 2002; Coltman & Slate 2003). The critique was based on the fact that mean d^2 was found to be poorly correlated with the inbreeding coefficient in a small population of captive wolves (*Canis lupus*; Hedrick *et al.* 2001) and under several simulated population scenarios (Tsitrone *et al.* 2001). Tsitrone *et al.* (2001) modelled both partial selfing and admixture, and showed that heterozygosity produced stronger genotype–fitness correlations than d^2 except under certain restricted conditions. Only when very large and recently intermixed populations were studied with microsatellites with high mutation rates, d^2 was found to provide a slightly better estimate of the inbreeding coefficient and fitness than a plain heterozygosity measurement (Tsitrone *et al.* 2001). In contrast, many significant mean d^2–fitness correlations had been detected in small populations studied with at least some less variable microsatellites. Consequently, it was concluded that mean d^2 provides less information of inbreeding and outbreeding in most natural situations and microsatellite mutation scenarios than does MLH (Hedrick *et al.* 2001; Tsitrone *et al.* 2001), and that published mean d^2–fitness correlations might be spurious type I errors with no or little biological relevance (Tsitrone *et al.* 2001; Goudet & Keller 2002).

Despite this explicit and strong critique, the mean d^2-approach is still being frequently used in conservation and evolutionary biology (e.g., Marshall *et al.* 2003; Borrell *et al.* 2004; Hansson *et al.* 2004; Neff 2004; Lesbarreres *et al.* 2005; MacDougall-Shackleton *et al.* 2005; Da Silva *et al.* 2006, 2009; Kretzmann *et al.* 2006; Zachos *et al.* 2007; Andersen *et al.* 2008; Fratini *et al.* 2008; Lie *et al.* 2008; White & Searle 2008; Fossoy *et al.* 2009). In light of the discrepancy between theory and practise, it is relevant to critically re-evaluate the mean d^2-approach, compare it with the MLH-approach, discuss the relevance of alternative hypotheses other than those based on identity disequilibrium, and examine whether there might be certain features of the mean d^2-parameter, the microsatellites or the study populations that may explain the occurrence of the mean d^2–fitness correlations.

The role of highly variable loci

Several researchers have suggested that mean d^2 may be a better predictor of fitness than MLH when highly variable microsatellites are used (e.g. Marshall *et al.* 2003; Kretzmann *et al.* 2006). One of the most obvious differences between MLH and mean d^2 is that markers of different variability contribute differently to the total variance of these measurements. The variance in single-locus d^2, and SLH (single-locus heterozygosity), respectively, in a population will differ markedly for microsatellites with little, intermediate and high variability: the variance in d^2 increases with increasing variability, whereas the variance in SLH reaches an optimum for microsatellites with intermediate variability. In other words, mean d^2, but not MLH, provides a scaling in favour of highly variable loci. In Fig. 1, I exemplify these patterns at 19 microsatellites in a population of great reed warblers (*Acrocephalus arundinaceus*). As expected, there is a strong positive, almost exponential, relationship between the standard deviation (i.e. the square root of the variance) of d^2 and the locus-specific heterozygosity in the population, whereas there is a humped shaped relationship for SLH (Fig. 1; data from Hansson *et al.* 2004; B. Hansson, unpublished).

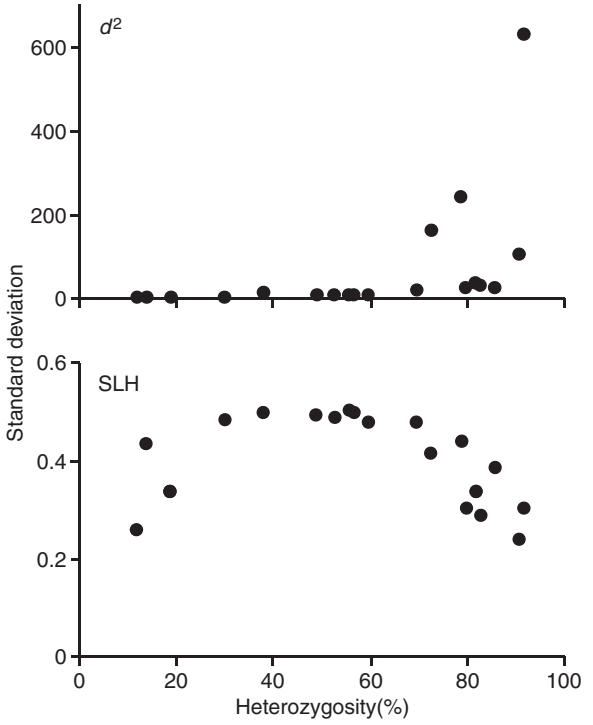

Fig. 1 The standard deviation of single-locus d^2 and heterozygosity (SLH) in a Swedish population of great reed warblers plotted against the locus-specific variability at 19 microsatellite loci (data from Hansson *et al.* 2004; B. Hansson, unpublished). Locus-specific variability is given as average heterozygosity in the population (Hansson *et al.* 2004).

This implies that when markers with different variability are being used, the mean d^2-value of an individual will be almost entirely determined by a few highly variable loci, whereas the MLH-value will be determined by a larger number of loci with intermediate heterozygosity. From this follows that if highly variable loci for some reason were more informative of the fitness-associated trait, mean d^2 may outperform MLH in genotype–fitness correlations. This scenario further predicts that measurements of mean d^2 which have been standardized for the locus-specific variance in the population will be less informative than the un-standardized ones (cf. Coulson *et al.* 1999; Höglund *et al.* 2002).

One reason to why highly variable loci may perhaps provide a better prediction of fitness-associated traits is that the mean d^2-approach is highly dependent on stepwise mutations to create the temporal memory in the allelic distance data (i.e. alleles of similar length are more likely related by descent than alleles of different length) that can be utilized to estimating inbreeding coefficients (Coulson *et al.* 1998, 1999; Pemberton *et al.* 1999). Here, it is plausible that highly variable microsatellites play a key role, because loci with many repeats and wide range in allele size are especially likely to follow the SMM (Rose & Falush 1998; Schlötterer 2000; Lai & Sun 2003). According to this scenario, a positive association between locus-specific variability and the strength of the d^2–fitness correlation would be predicted, whereas this association should be weaker or absent for SLH. However, in two studies that present single-locus data for both diversity measurements, mean d^2 did not correlate stronger to fitness at highly variable microsatellites than did MLH. In the great reed warbler (Hansson *et al.* 2004), the correlation between locus-specific heterozygosity (range: 0.21–0.96; n = 19 loci) and the effect size of the d^2–fitness correlation (r_S = 0.52) was very similar to that of SLH (r_S = 0.47); and the effect sizes of the two measurements were highly correlated (r_S = 0.89). In Chinook salmon (*Oncorhynchus tshawytscha*; Heath *et al.* 2002), the corresponding correlations between locus-specific heterozygosity (range: 0.69–0.92; n = 7 loci) and the strength of the d^2–fitness correlation, and SLH–fitness correlation, were r_S = 0.16 and r_S = 0.22, respectively. Thus, there was no support for the idea that the use of highly variable loci exclusively improves the mean d^2–fitness correlation; if anything, these results indicate that highly variable markers improve both types of genotype–fitness correlations and do not support the usage of the mean d^2-approach.

Tight linkage and linkage disequilibrium

An alternative explanation for genotype–fitness correlations, which was considered neither by Coulson *et al.*'s hypothesis (Coulson *et al.* 1998) nor the critique (Tsitrone *et al.* 2001), is that microsatellites may be located in gene-dense regions or that there is extended linkage disequilibrium in the population, and therefore that the potential for 'local effects' is much stronger than previously suspected. The question is whether this would help explain why mean d^2 may outperform MLH as a fitness-trait predictor?

The local effect hypothesis suggests that markers in linkage disequilibrium (LD) with linked fitness genes reflect the diversity of these genes and reveals selection acting upon them. This selection could either act upon recessive partly deleterious alleles or be visible as heterozygous advantage (David 1998; Hansson & Westerberg 2002). Accordingly, an individual that is homozygous or has low mean d^2 at the markers is homozygous also at one or more linked causative gene (or, strictly, one or more genes in LD with the marker), which in turn reduces fitness. Here, variation in MLH or mean d^2 between individuals can be generated by random segregation as well as by inbreeding. Thus, genotype–fitness correlations could appear as one happens to use markers located in the same chromosomal region as genes determining the trait under study.

Local effects depend heavily on LD, because the markers will be informative of more genes when there is much LD. So how much LD do we expect in natural populations? LD can be generated in small populations due to drift, during admixture of differentiated populations, and in recently bottlenecked-and-expanded populations due to the rapid increase in number of few (not yet recombined) haplotypes. Moreover, spatially and temporally varying selection pressures, and selection for co-adapted gene complexes, increase LD, and this has been suggested to cause strong LD in some species (e.g., side-blotched lizard, *Uta stansburiana*; Sinervo & Clobert 2003; see also Ford-Lloyd *et al.* 2001). Recombination will effectively break down LD; leaving populations that have been large and panmictic for a long period in linkage equilibrium (Chakraborty & Weiss 1988; Hartl & Clark 1997; Iles & Bishop 1998). Interestingly, the recombination rate varies throughout the genome and between species (e.g. Nachman & Churchill 1996; True *et al.* 1996; Winckler *et al.* 2005; Dawson *et al.* 2007), and this complexity is now starting to be understood. In human, non-randomly distributed recombination hotspots result in chromosome blocks with little recombination and high LD (Goldstein 2001; Reich *et al.* 2001). However, these LD-blocks are small (often less than 10 kb); too small to be of general importance in the context of genotype–fitness correlations. Thus, as most theoretical assessments of LD suggest, it is likely that there are relatively low levels of

LD in most natural populations (Chakraborty & Weiss 1988; Hartl & Clark 1997; Iles & Bishop 1998) and this view is corroborated by the results from several species (e.g. Dunning *et al.* 2000; Backström *et al.* 2006; Cutter *et al.* 2006; Balakrishnan & Edwards 2009).

Nevertheless, convincing support for the local effects hypothesis comes from a few within-family (i.e., within-inbreeding coefficient) analyses (Leary *et al.* 1987; Bierne *et al.* 1998; Hansson *et al.* 2001, 2004; Markert *et al.* 2004; Bensch *et al.* 2006). In these studies there were positive associations between the trait and heterozygosity, even if there was no variation in the inbreeding coefficient (i.e., ruling out possibilities for an effect at genome-wide distributed fitness loci; Hansson *et al.* 2004; Hansson & Westerberg 2008). However, in light of the predicted and observed weak LD in many populations, it seems too optimistic to imagine that LD should hold as a general explanation for genotype–fitness correlations; it may only apply to certain populations with exceptional LD generated by admixture or bottlenecks (e.g. Hansson *et al.* 2004; Bensch *et al.* 2006), or certain experimental setups reinforcing strong LD (e.g. Bierne *et al.* 1998). Moreover, in neither of the studies where extended LD due to recent bottleneck–and–expansion have resulted in local effects, did mean d^2 improve the genotype–fitness association (Hansson *et al.* 2004; Bensch *et al.* 2006; S. Bensch & M. Åkesson, personal communication).

Could it be that microsatellites are non-randomly distributed and generally found very close, or even within, functional genes? Then, even the small-scaled levels of LD that are generally occurring in natural populations (e.g. Dunning *et al.* 2000; Backström *et al.* 2006; Cutter *et al.* 2006; Balakrishnan & Edwards 2009) could result in local effects. In fact, microsatellites of a certain type, the trinucleotide repeats, are more prevalent in exons than previously suspected (Toth *et al.* 2000; Ellegren 2004). However, the majority of microsatellites, and including non-trinucleotide repeats, are distributed over the genome and mainly found in non-coding DNA, such as intergenic regions and introns (Toth *et al.* 2000; Ellegren 2004). This, together with the difficulties with which candidate genes are detected even in systematic searches in large-scale controlled crossing experiments, suggest that it would be unlikely that a few more or less randomly picked markers would be closely linked to or even embedded in important fitness genes. Still, it is difficult to completely rule out this possibility and there exist examples where a small set of markers has included single markers that have been tightly linked to important fitness genes (e.g. *Ase46* in magpies *Pica pica*, Martin-Galvez *et al.* 2006; see also Hanski & Saccheri 2006 and Luikart *et al.* 2008).

Finally, it is possible that some microsatellites are directly involved in trait expression, i.e. have 'direct effects', as is the case for some human neurodegenerative disorders (e.g. fragile X syndrome and Huntington's disease; reviewed in Bates & Lehrach 1994). Interestingly, in such particular cases several assumptions of the mean d^2-approach are potentially fulfilled (but this needs to be verified), including high marker variability, step-wise mutations, selection against long alleles and fitness effects. However, such direct fitness effects should apply to an absolute minority of microsatellites, and does not serve as a general explanation for genotype–fitness correlations. Studies that are simultaneously evaluating the fitness effects at single loci (e.g. Coulson *et al.* 1999), the genome location of markers (e.g. Bensch *et al.* 2006), the degree of LD (e.g. Bensch *et al.* 2006) and include candidate genes (e.g. Luikart *et al.* 2008; Da Silva *et al.* 2009) will provide important contributions towards the understanding of local and direct effects.

Admixture and genome-wide effects

As pointed out in the original hypothesis for the mean d^2-approach (Coulson *et al.* 1998, 1999; Pemberton *et al.* 1999), admixture of differentiated populations is a main candidate explanation to why mean d^2 sometimes seems to result in a stronger fitness correlation than MLH. According to this scenario, mean d^2 captures information about the immigration history of individuals that is not covered by a plain heterozygosity measure. Many species are patchily distributed in populations of varying sizes and genetic drift may cause population differentiation (the 'Wahlund effect'). Drift and bottlenecks will in particular cause allele frequency differences and loss of alleles at highly variable loci (Luikart *et al.* 1998a,b). Over the geographical range of a species, various more or less complex admixture events may occur, e.g. asymmetric dispersal from large populations to small population, or exchange between small populations. In such metapopulation systems, mean d^2 can be hypothesized to be better at detecting admixture than MLH, because at highly variable loci most individuals will be heterozygous whereas immigrants and/or their 'hybrid' descendants will on average have a higher mean d^2. If the latter ones are fitter on average due to heterosis, mean d^2–fitness correlations would be stronger than the corresponding MLH–fitness correlations. Indeed, signs of admixture due to introductions of animals from several distinct source populations were most likely what Coulson *et al.* (1998, 1999) detected in their studies of birth weight and first-year overwinter survival in red deer calves.

Admixture has also often been invoked in the studies following the publication of Tsitrone *et al.*'s (2001) critique of the mean d^2-approach. As mentioned above, although d^2 was found to be a poor estimate of inbreeding in most model scenarios, it provided a slightly better estimate of the inbreeding coefficient and fitness than heterozygosity when large and recently intermixing populations were studied with highly mutable markers (Tsitrone *et al.* 2001). For example, admixture was recently suggested by Fratini *et al.* (2008) to explain a mean d^2–fitness association in the intertidal crab *Pachygrapsus marmoratus*, by Kretzmann *et al.* (2006) in harp seals (*Phoca groenlandica*), and by Neff (2004) in a bluegill sunfish (*Lepomis macrochirus*) population. Interestingly, in the sunfish study, individuals were spread along the inbreeding–outbreeding continuum due to occasional kin-mating as well as hybridization with a closely related species, and, accordingly, there was a curved relationship between mean d^2 and survival; individuals with either low (inbred) or high (hybrids) mean d^2 had lower fitness than individuals with intermediate mean d^2. This pattern could not have been detected with a univariate model between fitness and a plain heterozygosity measurement. However, it is possible that a multivariate model that included the interaction term between MLH and a hybrid category would have resulted in a similar conclusion as for the mean d^2–fitness correlation, but with the additional advantage of gaining a straightforward interpretation of the actual cause of the genotype–fitness correlation (i.e. inbreeding and hybridization). Thus, evaluating the degree of population structure and dispersal between populations, using assignment tests to reveal immigrants (e.g. Beaumont *et al.* 2001; Sanz *et al.* 2009), and applying easily interpretable diversity measurements such as MLH, promise to lead to a better understanding of the roles of individual genetic diversity and population history, respectively, in the context of conservation and evolutionary genetics, than would a continuous use of the mean d^2-approach. When using the mean d^2-approach, increased understanding of the effects of recent and deeper inbreeding events may be reached by partitioning the variance into that explained by homozygosity (recent inbreeding events) and by mean d^2 at strictly heterozygous loci (deep outbreeding events; termed mean $d^2_{\text{outbreeding}}$ in Coulson *et al.* 1999).

Conclusions

I have discussed the possibility that certain features of the mean d^2 parameter (e.g. scaling favouring highly variable loci), the microsatellites (e.g. linkage with fitness-genes and functionality *per se*) or the study population (e.g. extended linkage disequilibrium and recent admixture) that were not fully considered by the critique (Tsitrone *et al.* 2001) may improve the mean d^2–fitness correlations. Among these potential explanations, two may be relevant in particular situations. First, some microsatellites (but still a minority), especially the trinucleotide repeats, are located in exons and could have a direct effect on fitness; and, at the same time, may fulfil several assumptions of the mean d^2-approach (e.g. high marker variability, step-wise mutations, selection against long alleles and fitness effects). Second, deep outbreeding due to admixture of differentiated populations is a main candidate to explain mean d^2–fitness associations (e.g. Coulson *et al.* 1998, 1999; Neff 2004; Kretzmann *et al.* 2006; Fratini *et al.* 2008), but only when very large and recently intermixed populations are studied with microsatellites with high mutation rates, and, even then, mean d^2 provides only a slightly better estimate of the inbreeding coefficient and fitness than MLH (Tsitrone *et al.* 2001). It seems unlikely that these explanations would be sufficient for all mean d^2–fitness correlations, and an alternative explanation, a publication bias in favour of significant mean d^2–fitness correlations, can not be ruled out (cf. Tsitrone *et al.* 2001; Goudet & Keller 2002). For example, it is possible that the mean d^2-approach gives weight to a few individuals that happen to have extreme inter-allelic distances at highly variable loci, and that the genotype–fitness correlation would be too dependent on the phenotypic and reproductive characteristics of these very few individuals with inflated statistics and conclusions as a result. Moreover, a major caveat is that it is difficult to predict a priori when (if indeed ever) mean d^2 will improve the genotype–fitness correlation, which in turn makes it problematic to design hypotheses and objectively interpret results. Mean d^2–fitness associations are potentially interesting, but the fact that we cannot easily understand them is problematic and should be thoroughly addressed in each study. Therefore, instead of hastily reached interpretations of mean d^2–fitness correlations, conclusions need support from complementary analyses, e.g. verifying close linkage between markers and fitness genes or admixture of genetically structured populations. Such analyses are aided by the wealth of genomic information, high-throughput genotyping arrays and powerful population genetics analytical tools now available.

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The author is an assisstant professor at Lund University interested in conservation genetics and evolutionary biology in general, and in the causes and consequences of inbreeding, the genetics and dynamics of evolutionary important traits, and the genetics of ecological speciation, in particular.
