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The problem of measuring trait-preference correlations without disrupting them.

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Abstract

A key element at the heart of the Fisher-Lande-Kirkpatrick model of the sexual selection process is the genetic correlation between (male) trait and (female) preference. The strength of this association is critical in determining a population's evolutionary trajectory, which is why estimating its magnitude is so important. In the Lande model, the trait-preference correlation is solely established and maintained by mate choice, and although it is unclear how exclusively mate choice does this in nature, the experimental designs typically employed to measure trait-preference genetic correlations could be systematically weakening estimates by not allowing free mate-choice (similarly with husbandry practices). The precise impact of the problem is unknown, and possibly unknowable, but simple solutions can be applied to ensure the accuracy of trait-preference correlation estimates is maximized.

Sexual selection is a major mechanism of organic evolution that can include both inter- and intra-sexual processes. For the former, the logic outlined by Fisher (1930), and formally modeled by Lande (1981) and Kirkpatrick (1982), has been called the null model of intersexual selection (Prum 2010). One key element at the heart of the Fisher-Lande-Kirkpatrick model is the genetic correlation between (female) preference and (male) trait. The strength of this correlation in part determines the likelihood of runaway selection, a scenario in which trait and preference become increasingly and exponentially extreme, but even without runaway, the trait-preference genetic correlation is fundamental in determining a population's evolutionary trajectory (Lande 1981; Arnold 1983).

Genetic correlations occur through two mechanisms, pleiotropy – where a gene influences more than one trait - and linkage disequilibrium – which is non-

55 random allele associations at two or more loci (Roff 1997). For the standard
56 model of the Fisher process, and as Lande (1981) established formally, linkage
57 disequilibrium underpins male-female trait-preference correlations. Importantly,
58 this comes about behaviorally rather than through other factors like the location
59 of genes within the genome – for example linkage disequilibrium can arise from
60 low recombinational probability between physically adjacent (i.e. linked genes)
61 or low mutation rates (so allelic variants are found together more often than
62 expected). However, in the current context linkage disequilibrium (non-random
63 association of trait-preference genes) is caused and maintained by mate
64 preference. Basically, females with preference for large (small) traits mate with
65 males having large (small) traits and thus offspring inherit trait-preference
66 combinations and the preference-trait genetic correlation is established.

67 Testing for the preference-trait genetic correlation is important (Bakker &
68 Pomiankowski 1995; Sharma et al. 2017), because it sits at the heart of the null-
69 model of sexual selection (Prum 2010), and if non-existent, a complete rethink of
70 evolution through sexual selection would arguably be required. However,
71 despite many studies documenting significant genetic variation for male traits
72 and female preferences (e.g. Simmons 1987; Houde 1997; Tomkins & Simmons
73 1999; Ritchie 2000; Morrow & Gage 2001; Blanckenhorn & Hosken 2003; Taylor
74 et al. 2007; Narraway et al. 2010; reviewed in Prokuda & Roff 2014), evidence
75 for significant trait-preference genetic correlations is limited (Greenfield et al.
76 2014). One contributing reason for this paucity is that most empirical
77 investigations of the genetic correlation have been seriously underpowered
78 (Sharma et al. 2017). In fact, most published studies have used data sets an
79 order of magnitude smaller than power analyses suggest may be necessary given
80 reasonable assumptions about the likely effect sizes they are trying to detect
81 (Sharma et al. 2017). There is however an additional problem that seems to
82 been largely overlooked (but for important exceptions see Bakker &
83 Pomiankowski 1995, and Bakker 1999), and that is that the classical breeding
84 designs most commonly used to test for genetic correlations remove the
85 influence of female preference on realized matings – they do not allow mate-
86 choice to occur. As a consequence, these designs are likely to disrupt

behaviorally established genetic linkage disequilibrium, and so weaken the male-female genetic correlation that is the very target of the study (and see Lynch & Walsh 1998 pp. 150-153).

Genetic correlations between traits are typically estimated from a pedigree structure (e.g. Hoffman 1999; Tomkins & Simmons 1999; Wagner et al. 1999; Taylor et al. 2007; reviewed in Falconer 1981; Roff 1997) created by randomly allocating dams to sires (e.g. a full-sib/half-sib design: Falconer 1981; Roff 1997; Lynch & Walsh 1998). Phenotypic data collected on the offspring from known families are then analysed (Falconer 1981; Roff 1997; Lynch & Walsh 1998). When genetic correlations are caused by pleiotropy or close physical linkage (where recombination is unlikely to break up non-random gene associations), then these designs are perfectly appropriate. However, in the case of behaviorally generated linkage disequilibrium, which is expected to underpin Fisherian sexual selection (Fisher 1930; Lande 1981), a mating structure (i.e. pedigree) imposed by the experimenter has the effect of scrambling the naturally-occurring behaviorally-generated linkage disequilibrium that emerges from free mate choice (over and above any recombination effects). The same scrambling can also be generated if animal husbandry does not allow free mate-choice.

This can be illustrated with a simple 2-allele 2-locus thought experiment, and for simplicity assume a haploid organism, where one locus is for the male trait and one for female preference: where *T* is a large male trait, *t* a small male trait, *P* female preference for a large trait and *p* preference for a small trait. When animals are free to choose, and realized matings perfectly reflect preference we get total linkage disequilibrium between *T* and *P* and *p* and *t*. All offspring generated are *TP* or *tp* offspring and there is a strong genetic correlation between trait and preference. However, with random mate allocation by an experimenter, all four possible genotypes, *TP, Tp, tP*, and *tp,* are generated in the offspring. In other words the genetic correlation built up by behaviorally generated linkage disequilibrium under mate choice is weakened, and our ability to detect it will be reduced as a consequence. Note that we are not implying this

simple situation actually occurs in nature, merely using this for illustrative purposes of why the issue arises (also see Figure 1).

More formally, the importance of non-random mating can be seen using a simple approximation (Bakker & Pomiankowski 1995) for the predicted genetic correlation that is expected to build up via the Fisher process (Fisher 1930; Lande 1981). The equilibrium male-female genetic correlation (NB the covariance just due to mate preference) can be approximated as:

$$\mathrm{r}G \approx aG_{\mathrm{pref}}.G_{\mathrm{trait}}$$

where rG = the correlation, G_{pref} is the genetic variance for female preference, G_{trait} is the genetic variance for the male trait and a = strength of non-random mating (Bakker & Pomiankowski 1995). If the strength of non-random mating is weakened, the genetic correlation between trait and preference is weakened and if there is completely random mating (a = 0), there will be no correlation.

Complete randomness is hard to achieve and therefore unlikely in most studies, but experimenter assignment of mates could plausibly be making detection of significant trait-preference correlations much more difficult. Linkage disequilibrium breaks down for physically unlinked loci by 50% per generation (note this can be exceeded under special conditions: Lynch & Walsh 1998) when mate choice is disrupted for example (Bakker & Pomiankowski 1995) (Figure 1), and across all loci, rates of decay of the behaviorally generated linkage disequilibrium across the genome will depend on the number of generations that mate choice is disrupted and the recombination rate (see Lynch & Walsh 1998 pp 151 for a more mathematical description). This break-down could help to explain the paucity of significant trait-preference associations found empirically (Greenfield et al. 2014), particularly because the detection problem will be amplified by the low power of designs typically used (Sharma et al. 2017). It also means that there may be a systematic downward bias in published estimates of the trait-preference genetic correlation strength. The magnitude of the downward biasing will obviously depend on how much the trait-preference genetic correlation is due to linkage disequilibrium maintained by mate choice and recombination frequency. If the latter is small (0.001), then effects are

149 negligible, while if larger (0.1), breeding and husbandry designs could have large
150 effects on covariance estimation (Figure 2) (and see Lynch & Walsh 1998 pp. 96).

151 It is unclear just how big this problem is, but the detailed genetic architecture of
152 the traits will matter (chromosome number and loci locations, are they
153 Mendelian or polygenic, how many loci, what is the effect size distribution of
154 segregating alleles etc). We also don't know how much of existing trait-
155 preference genetic correlations (assuming they exist) is actually caused
156 behaviorally. It may be, for instance, that genome architecture evolves to
157 physically link trait-preference genes following initial establishment of
158 disequilibrium by mating preference. This would reduce recombination rates
159 and the impacts of random mate assignment. At present we have an incomplete
160 understanding of all these issue and it would be useful to explicitly explore some
161 of them. Nevertheless, the problem of random mate allocation in experimental
162 designs is clear, even if its (quantitative) consequences are not.

163 There are solutions for this experimental problem, and one obvious one is the
164 use of isofemale lines. Isolines are snapshots of naturally occurring genetic
165 variation and covariation (David et al. 2005) and can provide good estimates of
166 these parameters if tested appropriately (for full discussion see Hoffmann &
167 Parsons 1988). Experimenters could also allow test animals to freely choose
168 mates when establishing pedigrees to facilitate the establishment of behavioural
169 linkage disequilibrium between preference and trait. This presents no major
170 analytical problems provided the relationship structure among offspring can be
171 resolved *post hoc*, as the quantitative genetics of complex pedigrees can be
172 addressed using the animal model (Wilson et al. 2010).

173 The importance of behavioural linkage disequilibrium in maintaining intersexual
174 genetic correlations in sexual selection is clear in evolutionary models and logic
175 (Fisher 1930; Lande 1981). But is it important in empirical systems? The logic
176 suggests it must play a role in the establishment and maintenance of male trait-
177 female preference genetic correlations. Clearly this does not mean close physical
178 linkage and/or pleiotropy never contribute to these correlations, but we cannot
179 (implicitly) assume the opposite in our experimental approach either. Certainly,

some studies of individual genes have not yielded compelling evidence for pleiotropy. For example, *period* affects *Drosophila* courtship song but not female preference for it (Greenacre et al. 1993).

We suggest, as noted by others previously (Bakker & Pomiankowski 1995), that experimental disruption of trait-preference genetic correlation has largely been overlooked. We freely acknowledge that we have little general feeling for how much mate preference contributes to these correlations, and so little understanding of how big a problem this may be. This note is simply to raise awareness of these issues once more amid a resurgence of discussions about preference-trait associations, their importance and how to adequately test for them (e.g. Prum 2010; Prokop et al. 2012; Greenfield et al. 2014; Sharma et al. 2017).

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Figure 1. For unlinked loci linkage disequilibrium can decay at 50% per generation (possibly more under very restrictive conditions: Lynch & Walsh 1998). Shown here are two non-homologous chromosomes (grey and white lines) with a preference locus (P - only a single allele shown) on the grey chromosome and a trait locus (with T and t as two allelic alternatives) on a different (white) chromosome. With perfect assortative mate choice P and T are always found together (100% of the time) despite being on non-homologous chromosomes, but with random mate assignment P is found half the time with T and half the time with t – linkage between P and T decays by 50% with one generation of random mate assignment (and see Figure 2). Across all loci, linkage disequilibrium maintained by mate choice will be disrupted by random mating, such that proportion of covariance between sons and daughters (the genetic correlation due to mate choice) retained will depend on recombination rates (normally varying between 0-50%) and the number of generations without mate choice.

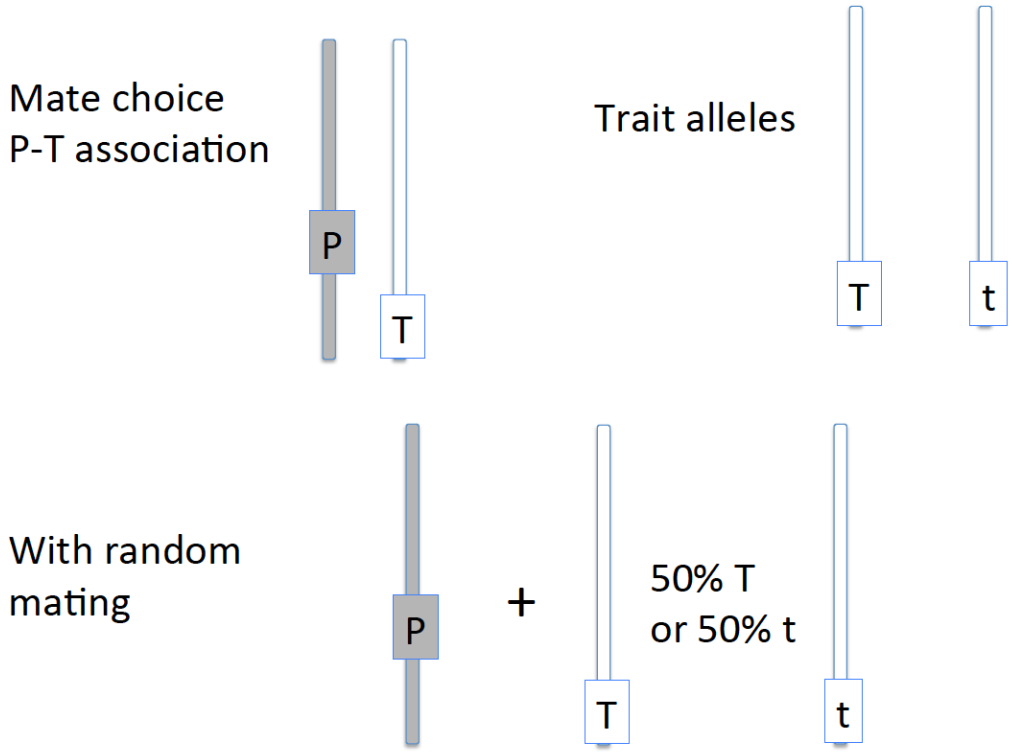

Figure 2. The decay rate of linkage disequlibrium under one (dotted line) or two generations (solid line) of random mating with various degrees of recombination. Linkage disequilibrium decays as: $(1-c)^g$, where c = the recombination rate and g = generation number (Lynch & Walsh 1998, pp 151). Thus when recombination frequencies are low (toward the origin), random mate assignment has little effect on linkage disequilibrium, but as recombination frequencies increase, effects become considerable. If trait-preference loci are largely unlinked and non-random mating is a major cause of trait-preference correlation (two unknowns), current experimental designs will seriously under-estimate the magnitude of trait-preference covariance. Multigenerational designs and animal husbandry without mate choice will magnify effects given the longer time scales involved.