PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Tuni C, Han CS, Dingemanse NJ. 2018 Multiple biological mechanisms result in correlations between pre- and post-mating traits that differ among versus within individuals and genotypes. *Proc. R. Soc. B* **285**: 20180951.
http://dx.doi.org/10.1098/rspb.2018.0951

Received: 27 April 2018
Accepted: 26 July 2018

Subject Category:
Ecology

Subject Areas:
behaviour, ecology, evolution

Keywords:
trait correlations, genetic quality, ejaculate expenditure, life-history trade-offs, male contest competition

Authors for correspondence:
Cristina Tuni
e-mail: cristina.tuni@bio.lmu.de
Chang S. Han
e-mail: hcspol@gmail.com

†These authors contributed equally.

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.4201976.

THE ROYAL SOCIETY PUBLISHING

Multiple biological mechanisms result in correlations between pre- and post-mating traits that differ among versus within individuals and genotypes

Cristina Tuni[1,†], Chang S. Han[1,2,†] and Niels J. Dingemanse[1]

[1]Behavioural Ecology, Department of Biology, Ludwig-Maximilians University of Munich, Planegg-Martinsried, Germany
[2]The School of Biological Sciences, Seoul National University, Seoul, South Korea

CT, 0000-0002-7190-1143; CSH, 0000-0001-9261-4031

Reproductive traits involved in mate acquisition (pre-mating traits) are predicted to covary with those involved in fertilization success (post-mating traits). Variation in male quality may give rise to positive, and resource allocation trade-offs to negative, covariances between pre- and post-mating traits. Empirical studies have yielded mixed results. Progress is hampered as researchers often fail to appreciate that mentioned biological mechanisms can act simultaneously but at different hierarchical levels of biological variation: genetic correlations may, for example, be negative due to genetic trade-offs but environmental correlations may instead be positive due to individual variation in resource acquisition. We measured pre-mating (aggression, body weight) and post-mating (ejaculate size) reproductive traits in a pedigreed population of southern field crickets (*Gryllus bimaculatus*). To create environmental variation, crickets were raised on either a low or a high nymphal density treatment. We estimated genetic and environmental sources of correlations between pre- and post-mating traits. We found positive genetic correlations between pre- and post-mating traits, implying the existence of genetic variation in male quality. Over repeated trials of the same individual (testing order), positive changes in one trait were matched with negative changes in other traits, suggesting energy allocating trade-offs within individuals among days. These findings demonstrate the need for research on pre- and post-mating traits to consider the hierarchical structure of trait correlations. Only by doing so was our study able to conclude that multiple mechanisms jointly shape phenotypic associations between pre- and post-mating traits in crickets.

1. Introduction

Sexual selection operates both pre- and post-mating [1]. Evolutionary theory predicts that male reproductive traits involved in mate acquisition (pre-mating traits), such as ornaments and armaments, covary with those involved in fertilization success (post-mating traits), such as testes and sperm phenotype [2–5]. Understanding this phenotypic integration is important as depending on their association, selection acting on a pre-mating trait (e.g. weaponry) can potentially constrain or facilitate the evolution of a post-mating trait (e.g. ejaculate size) and *vice versa* [6–9]. Positive phenotypic associations between pre- and post-mating traits may arise from genetic variation in male quality or environmental variation in male body condition. Since maintaining sexual traits is energetically demanding, males of high genetic quality or males in good condition, may be able to invest substantially in *both* pre- and post-mating traits, leading to positive phenotypic correlations (e.g. between male attractiveness and ejaculate quality [10]). Female choice would thus favour male phenotypes with greater mating and fertilization abilities ('phenotype-linked fertility' hypothesis) [11]. Alternatively,

Table 1. Mechanisms shaping genetic (r_A), permanent environmental (r_{PE}) and within-individual environmental (r_E) correlations between pre- and post-mating traits.

	correlations of positive sign	correlations of negative sign
r_A	genetic variation in ability to simultaneously invest in multiple costly phenotypic traits (genetic quality) [23–25]	genetic variation in how trade-offs between investments in multiple costly phenotypic traits are resolved [26–28]
r_{PE}	non-genetic among-individual variation in resource acquisition affecting ability to simultaneously invest in multiple costly phenotypic traits	non-genetic among-individual variation in how trade-offs between investments in multiple costly phenotypic traits are resolved
r_E	non-genetic within-individual variation in resource acquisition affecting ability to simultaneously invest in multiple costly phenotypic traits	non-genetic within-individual variation in how trade-offs between investments in multiple costly phenotypic traits are resolved

negative phenotypic associations (e.g. between weapons and testes [12]) may arise due to genetic or environmental trade-offs in resource allocation, leading to alternative reproductive strategies among males that, consequently, invest either primarily in pre- or post-mating traits [13,14].

Researchers have, interestingly, found contrasting patterns across taxa, reporting positive, negative or no phenotypic correlations between pre- and post-mating traits [2,4]. We highlight here two key explanations for such discrepancies, both hinging on the notion that phenotypic correlations arise because of the joint effects of genetic, environmental and error correlations. We give a mathematical example to illustrate both points, focusing on the phenotypic correlation (r_P) between a pre-mating trait (A) and a post-mating trait (B) that were each measured once per individual. This phenotypic correlation is then influenced both by the genetic correlation (r_A) and the residual correlation (r_R) between the traits, the latter reflecting the joint influences of environmental and measurement error correlations (equation (1.1); [15]):

$$r_P = r_A\sqrt{h_A^2 h_B^2} + r_R\sqrt{(1-h_A^2)(1-h_B^2)}. \quad (1.1)$$

This equation, where h_A^2 and h_B^2 represent the narrow-sense heritability of traits A and B, illustrates that species differences in phenotypic correlations can arise from species-specific genetic correlations and/or from species-specific residual correlations between pre- and post-mating traits. Phenotypic correlations can also differ between species when neither genetic nor residual correlations differ between species. This latter explanation requires level-specific correlations ($r_A \neq r_R$) in combination with species differences in geometric mean heritability ($\sqrt{h_A^2 h_B^2}$). Level-specific correlations have repeatedly been demonstrated by quantitative geneticists [16–18]. Importantly, negative genetic correlations indicative of trade-offs are often not be visible in phenotypic data owing to positive residual correlations resulting from individual differences in resource acquisition [19,20]. Equation 1 thereby illustrates the importance of decomposing phenotypic correlations between hierarchical levels [21]. Doing so is particularly useful when biological hypotheses concern specific hierarchical levels [22]. For example, the hypothesis that pre- and post-mating traits covary because of genetic variation in male quality predicts the existence of positive genetic—not simply phenotypic—correlations between pre- and post-mating traits. Equation (1) also illustrates the notion that multiple mechanisms, acting at different hierarchical levels, can jointly shape phenotypic associations between pre- and post-mating traits. This study, therefore, used a quantitative genetics approach to estimate the relative importance of trade-offs versus variation in male quality as genetic and environmental drivers of phenotypic correlations between pre- and post-mating traits.

Pre- and post-mating traits, like aggressiveness, body mass or sperm number represent phenotypic traits that are repeatedly expressed and thus vary both within and among individuals. Negative versus positive environmental correlations reflective of trade-offs in resource allocation versus variation in male condition can, therefore, also exist both among and within individuals. For example, variation in resource availability among individuals during development may cause long-lasting individual differences, estimable as positive permanent environmental correlations (r_{PE}; among-individual correlations between pre- and post-mating traits that are of non-genetic origin, table 1, [29]). Temporal variation in resource availability experienced by an individual repeatedly expressing pre- and post-mating traits may also lead to correlations across expressions of the same individual (i.e. *within* an individual). For example, on days where an individual is able to acquire many resources, it might upregulate its investment in both pre- and post-mating traits, while on days where it instead acquires few resources, it might downregulate its investment in both pre- and post-mating traits, leading to a positive within-individual environmental correlation due to within-individual plasticity integration (r_E, table 1). In other words, sexual selection studies focusing on repeatedly expressed pre- and post-mating traits should consider correlations at all three hierarchical levels (genetic, permanent environmental and within-individual, table 1) contributing to phenotypic correlations between pre- and post-mating traits (equation (1.2)):

$$r_P = r_A\sqrt{\frac{V_{A_A}}{V_{P_A}}\frac{V_{A_B}}{V_{P_B}}} + r_{PE}\sqrt{\frac{V_{PE_A}}{V_{P_A}}\frac{V_{PE_B}}{V_{P_B}}} + r_E\sqrt{\frac{V_{E_A}}{V_{P_A}}\frac{V_{E_B}}{V_{P_B}}}. \quad (1.2)$$

Here, V_P, V_A, V_{PE} and V_E represent the total phenotypic, additive genetic, permanent environmental, and residual within-individual variances, respectively, and V_A/V_P equals the h^2 in equation (1.1). Furthermore, r_E represents the within-individual correlation attributable to within-individual plasticity integration and correlated measurement errors.

The aim of this study was to estimate genetic and environmental correlations between male pre- and post-mating traits in the southern field cricket *Gryllus bimaculatus*. Male field crickets engage in pre-mating intra-sexual competition in the

form of aggressive male–male fights over control of breeding shelters (e.g. a protected burrow or crevice) [30,31]. Dominant contest winners are larger and sing from their shelters to attract females [32]. Females mate with multiple partners [33,34], causing intense post-mating sperm competition, known to induce greater allocation to post-mating traits such as sperm numbers [35,36] or viability [37]. Therefore, if pre- and post-mating traits both reflect male genetic quality, a positive genetic correlation is predicted (table 1). In addition, if within-individual changes in aggression (pre-mating trait) and sperm number (post-mating trait) are influenced by within-individual changes in resource acquisition (hence body weight), strong positive within-individual correlations among these three traits would be expected (table 1). By contrast, in the presence of trade-offs, negative genetic correlations between pre- and post-mating traits are expected (table 1), with male genotypes possessing larger ejaculates at the expense of body size and aggressiveness. For example, in the cricket *Acheta domestica*, small males are known to produce twice as much sperm compared to (dominant) males of higher body mass [38]. Likewise, strongly negative within-individual correlations between pre- and post-mating traits are predicted if trade-offs occur at the within-individual level. Of course, when mechanisms (quality dependence or trade-off) shaping correlations differ across hierarchical levels, the resulting phenotypic correlations are uninterpretable unless partitioned statistically (equations (1.1) and (1.2)).

We further expected that environmental correlations between pre- and post-mating traits would specifically be a function of social experiences during development. Cricket nymphs raised in environmental conditions mimicking a population with a high presence of males allocate more to reproductive tissue (testis and accessory glands) [39]. One of our recent studies on the southern field cricket suggests trade-offs in resource allocation between pre-mating (fighting ability) and post-mating (ejaculate quality) traits, because contest winners produced sperm of lower quality compared to losers [40]. Thus, to shed light on whether patterns of permanent environmental correlations among sexually selected traits were underpinned by variation in the developmental social environment, we additionally manipulated density during ontogeny. We exposed nymphs of an established pedigreed population to two rearing density conditions, namely 'low' and 'high' densities, and scored two pre-mating traits, aggressiveness during staged encounters with opponent males and body mass (known to affect fight outcome and female choice), and one post-mating trait, ejaculate size (sperm numbers), for these males in adulthood.

2. Material and methods

(a) Animal maintenance, breeding design and density treatments

Around 400 nymphs were collected from a large wild population of the southern field cricket in Tuscany (Italy) in July 2015, and transported to our laboratory. We housed them (and their offspring, described below) in climate rooms at 26°C with 40% relative humidity under a 14 L : 10 D photoperiod. Nymphs (approx. 20) were commonly placed in large plastic tanks ($23 \times 15 \times 17$ cm^3) furbished with pieces of egg carton for shelter, a plastic water vial plugged with cotton wool and dry bird food (Aleckwa Delikat, Germany). Nymphs developing into last instars were isolated individually in plastic containers ($10 \times 10 \times 9$ cm^3) with shelter, water and food. Once reaching adulthood, individuals were assigned to a maternal half-sib/full-sib breeding design [7] in which we randomly selected 47 unrelated males (sires) from the laboratory population and had each male consecutively fertilize the clutches of two unrelated virgin females (dams) (detailed in the electronic supplementary material).

Once eggs hatched, we adopted a split brood design, where full-sib nymphs were randomly assigned to one of two density treatments: a 'low density treatment' consisting of a total of seven full-sib nymphs, or a 'high density treatment' with a total of 20–25 full-sib nymphs, reared communally. In both treatments, nymphs were raised in a plastic tank ($23 \times 15 \times 17$ cm^3) provided with shelter, and ad libitum food and water (as above). To equalize sample sizes across treatments we maintained four low-density and two high-density tanks per full-sib family.

When nymphs reached the last instar, or eclosed to adults, we transferred males to a container ($10 \times 10 \times 9$ cm^3) equipped with shelter, water and food, and housed each cricket individually to control for mating and social experience until sexual maturation. Ten days after reaching adulthood we measured pre-mating traits (aggressiveness and body size) and a post-mating trait (sperm number) in adult males. All traits were measured four times per male with a 2-day interval.

(b) Measures of pre-mating traits: aggressiveness and body size

Male aggressiveness was scored during interactions with an opponent male following established protocols [41–44]. Prior to tests, all males were individually marked with a small dot of acrylic paint (Testors enamel paint) on their pronotum to ease individual identification. Tests consisted of random pairing of two unrelated males for a total duration of 5 min. Males were transferred from their individual containers to a plastic arena with a removable partition in the middle to separate two smaller compartments ($15 \times 15 \times 10$ cm^3). A single male was placed in each of the two compartments, and was allowed to freely move inside its compartment for a total of 5 min. Upon the start of the assay, the partition between the two compartments was removed, allowing the two males to interact. The arena was furbished with fine-grained white sand on the bottom to facilitate movements of animals. When male crickets of this species interact, they typically escalate from low-level aggression (e.g. antennal fencing, threat postures) to high-level aggression (e.g. aggressive song stridulation, flaring mandibles and biting) with one male, the winner, chasing and attacking a fleeing male, the loser [30].

All behavioural assays were video recorded and analysed using an automated tracking software (Noldus Ethovision XT 10, Noldus Information Technology) measuring the amount of time a focal individual spends chasing the opponent male (within a 6 cm distance). Based on previous work, this measure represents an appropriate proxy for aggressiveness in this species [44]. After each behavioural assay terminated, male body mass was determined to the nearest 0.001 g using a digital scale (KERN PKT), and males returned to their housing containers.

(c) Measures of a post-mating trait: ejaculate size

On the day prior to the behavioural assays each males' genital opening was checked and any spermatophore present was gently removed using soft forceps and discarded in order to standardize spermatophore age. On the following day, prior to each behavioural assay, a spermatophore was collected for sperm counts to determine ejaculate size. The spermatophore was placed into 200 µl of Beadle saline (128.3 mM NaCl, 4.7 mM KCl and 23 mM $CaCl_2$) and was left to release its contents for 10 min after the evacuating tube was cut with fine scissors. We estimated

the number of sperm present in the spermatophore using an automated cell counter (CASY Schärfe-System, Reutlingen, Germany) [45]. A 100 µl aliquot of the diluted sperm sample was added and mixed by inversions to 0.99 ml of CASY-ton solution, an isotonic and iso-osmotic electrolyte and the prepared vial was then inserted in the cell counter that estimates the total number of cells in the sample from three automatized consecutive measurements.

(d) Statistical analyses

We used a two-step approach to analyse our data. Our first set of analyses tested density treatment and testing order effects on trait means, for which we fitted univariate mixed-effects animal models [29,46]. Treatment effects on both pre- and post-mating traits would provide experimental evidence for a role of density during development causing permanent environment correlations, as treatment varied among individuals. Similarly, effects of testing order (i.e. due to experience) on both pre- and post-mating traits would provide empirical evidence for its role in shaping within-individual environmental correlations. Our second set of analyses quantified phenotypic, genetic, permanent environmental and within-individual correlation structures between pre- and post-mating traits, for which we fitted sets of multivariate animal models (detailed below) after combining the two datasets from different density treatments. Values of traits other than body weight were transformed to fulfil normality assumptions (aggression, log-transformed; sperm number, square-root-transformed). All the trait values were also z-transformed (mean = 0, s.d. = 1) prior to analyses. All models were implemented in ASReml (version 4, VSN interaction Ltd, Hemel Hempstead, UK), and solved using restricted maximum likelihood.

(i) Univariate mixed-effects models

We first analysed the effect of density treatment and testing order on trait mean and sources of variation in each phenotypic trait (aggression, body weight, ejaculate size) separately, using univariate animal models, where the focal trait was fitted as the response variable, and where density treatment (two-level factor: low versus high), testing order (covariate), testing shelf (two-level factor: lower versus upper; behavioural traits only) and time of the measurement (covariate) were fitted as fixed effects. Testing order and time of the measurement were mean-centred at the population level. Using the pedigree information, we partitioned the phenotypic (co)variances into additive genetic (V_A), permanent environment (V_{PE}) and nymph container identity effects (V_C), and residual within-individual (V_R). Here, permanent environmental effects (V_{PE}) were defined as the among-individual variance in repeated measures data not attributable to additive genetic (V_A) or container effects (V_C). In addition, only for aggression, the variance attributable to interacting partner ($V_{partner}$) was additionally estimated as appropriate [44,47].

(ii) Multivariate mixed-effects models

We fitted a multivariate animal model to estimate correlation structures at various levels (within-individual, genetic, among-individual and phenotypic levels) with the same fixed-effects structure as detailed above. The model fitted (1) aggression, (2) body weight and (3) ejaculate size as the three response variables, allowing us to measure the additive genetic variance–covariance matrix (G-matrix) utilizing the information embedded in the relatedness matrix [7,29,46]. In the model, we partitioned the phenotypic (co)variances into additive genetic (V_A) and permanent environment (V_{PE}) and residual within-individual (co)variance components. In addition, only for aggression, the effect of partner's identity ($V_{partner}$) was added as an additional random effect as appropriate [41,44]. Since the effect of the nymph container identity explained little (less than 5%) of the total phenotypic variation (as in our previous studies: [43] it was not included in the multivariate models, which facilitated model convergence. Level-specific correlations between traits (A and B) were calculated from their estimated level-specific variances (V) and covariances (Cov), where $r_x = \mathrm{Cov}_x / \sqrt{V_{x_A} V_{x_B}}$, and x represents the hierarchical level of interest. We added up all level-specific covariances to calculate the phenotypic covariance (Cov_P), and added up all level-specific variances for each trait separately to estimate each trait's phenotypic variance (V_{P_A}, V_{P_B}). We then calculated the phenotypic correlation (r_P) from those (co)variances as detailed for level-specific correlations above.

(iii) Significance testing

The significance of fixed effects was derived from conditional Wald F-tests. In addition, we assessed the significance of (within-individual, genetic, permanent environmental and phenotypic) variances/covariances/correlations using likelihood ratio tests (LRTs). The test statistic associated with the LRT was calculated as the χ^2-distributed difference in deviance (−2*loglikelihood) between the full model and a model where a focal random effect was removed. For tests of non-zero variances (applied to univariate models only), the value of P was calculated assuming an equal mixture of $P(\chi^2, \mathrm{df} = 0)$ and $P(\chi^2, \mathrm{df} = 1)$ [48,49] denoted as '$\chi^2_{0/1}$' in our statistical tables. In addition, to test whether a focal genetic covariance/correlation deviated from zero (applied to multivariate models only), we compared a fully unconstrained model with a model where the focal genetic correlation of interest was constrained to zero, and applied a LRT testing against P (χ^2, d.f. = 1).

3. Results

Males that had developed in low densities were heavier than males developed in high densities (table 2). By contrast, aggression and ejaculate size were not a function of the developmental density treatment (table 2). Thus, there were no widespread effects of nymph density environments on phenotypes, implying that experimental variation in density did not lead to any permanent environmental correlations indicative of developmental plasticity integration. Interestingly, males also became heavier, produced more sperm, and behaved less aggressively over the course the experiment (effect of testing order, table 2). Testing order affected all traits, implying that the three traits shifted over the course of the experiment (days) within single individuals.

All three traits were heritable (table 2). Genetic correlations between ejaculate size and aggression, and between ejaculate size and body weight, were all significant and positive (aggression–ejaculate size, r_A (s.e.) = 0.66 (0.19), $\chi^2_1 = 11.95$, $p < 0.001$; weight-ejaculate size, r_A (s.e.) = 0.43 (0.17), $\chi^2_1 = 10.41$, $p = 0.001$; table 3), providing strong empirical support for variation in male quality at the genetic level. However, body weight and aggression were not significantly correlated at the genetic level (r_A (s.e.) = 0.08 (0.18), $\chi^2_1 = 0.23$, $p = 0.63$; table 3). Permanent environmental correlations were either not different from zero (aggression–weight, r_{PE} (s.e.) = 0.41 (0.28), $\chi^2_1 = 3.04$, $p = 0.08$) or estimated at the boundary (table 3, electronic supplementary material, table S2). These bounded values were due to the absence of permanent environmental effects on aggression and sperm number (electronic supplementary material, table S2). Residual within-individual variances were significant for all traits (see electronic supplementary material, table S3). However, apart from the significant effect of testing order on trait means, there were no significant residual within-individual

Table 2. Effects of density treatment on mean values and sources of variation in aggression, body weight and ejaculate size (sperm number). χ^2 values were derived from LRTs (see Material and methods). V_A, additive genetic variance; V_{PE}, permanent environmental variance; V_C, variance explained by juvenile container identity; V_R, residual variance; $V_{PARTNER}$, variance explained by interacting partners; n.a., non-applicable.

	aggression			body weight			ejaculate size		
fixed effects	β (s.e.)	$F_{NUMdf,\ DENdf}$	*p*	β (s.e.)	$F_{NUMdf,\ DENdf}$	*p*	β (s.e.)	$F_{NUMdf,\ DENdf}$	*p*
intercept	0.06 (0.06)	$0.12_{1,50.0}$	0.73	−0.13 (0.09)	$0.40_{1,55.1}$	0.53	−0.02 (0.05)	$0.00_{1,55.2}$	0.96
density[a]	0.03 (0.06)	$0.24_{1,598.9}$	0.62	0.37 (0.07)	$31.73_{1,168.2}$	<0.001	0.04 (0.06)	$0.45_{1,150.7}$	0.50
testing order	−0.08 (0.02)	$11.57_{1,948.2}$	<0.001	0.02 (0.01)	$8.58_{1,2147.0}$	<0.001	0.20 (0.03)	$36.56_{1,969.2}$	<0.001
shelf	−0.18 (0.05)	$13.38_{1,1336.2}$	<0.001	n.a.			n.a.		
time of day	−0.03 (0.02)	$2.40_{1,1361.3}$	0.12	0.02 (0.01)	$21.20_{1,2211.5}$	0.004			
random effects	σ^2 (s.e.)	$\chi^2_{0/1}$ [b]	*p*	σ^2 (s.e.)	$\chi^2_{0/1}$	*p*	σ^2 (s.e.)	$\chi^2_{0/1}$	*p*
V_A	0.15 (0.05)	26.56	<0.001	0.50 (0.12)	84.26	<0.001	0.10 (0.04)	13.31	<0.001
V_{PE}	0.04 (0.04)	0.85	0.18	0.31 (0.07)	11.35	<0.001	0.00 (0.00)	0.00	0.50
V_C	0.00 (0.00)	0.00	0.50	0.05 (0.03)	3.53	0.03	0.02 (0.03)	0.95	0.16
$V_{PARTNER}$	0.10 (0.03)	0.002	<0.001	n.a.			n.a.		
V_R	0.69 (0.05)	—	—	0.09 (0.003)	—	—	0.85 (0.04)	—	—

[a]High density as contrast.
[b]An equal mixture of d.f. = 0 and d.f. = 1.

Table 3. Correlations at multiple hierarchical levels. Residual within-individual not attributable to fixed effects (r_E), genetic (r_A), permanent environmental (r_{PE}) and phenotypic correlations (r_P). Significant correlations ($p < 0.05$) are highlighted in bold face.

trait correlations	r_E	r_A	r_{PE}	r_P
aggression–weight	−0.04 (0.04)	0.08 (0.18)	0.41 (0.28)	**0.07 (0.03)**
aggression–ejaculate size	0.09 (0.07)	**0.66 (0.19)**	a	**0.18 (0.05)**
weight–ejaculate size	−0.02 (0.04)	**0.43 (0.17)**	a	**0.08 (0.04)**

[a]Variance components estimated at the lower boundary.

correlations among pre- and post-mating traits (aggression–ejaculate size, r_E (s.e.) = 0.09 (0.07), $\chi^2_1 = 1.06$, $p = 0.30$; aggression–weight, r_E (s.e.) = −0.04 (0.04), $\chi^2_1 = 1.92$, $p = 0.17$, weight–ejaculate size, r_E (s.e.) = −0.02 (0.04), $\chi^2_1 = 0.06$, $p = 0.81$; table 3). This indicated that the combined influence of all unmeasured environmental factors varying within individuals other than testing order (see above) did not cause additional within-individual correlations.

Finally, all phenotypic correlations were positive (aggression–weight, r_P (s.e.) = 0.07 (0.03), $\chi^2_1 = 6.52$, $p = 0.01$; aggression–ejaculate size, r_P (s.e.) = 0.18 (0.05), $\chi^2_1 = 9.34$, $p = 0.002$; weight–ejaculate size, r_P (s.e.) = 0.08 (0.04), $\chi^2_1 = 4.50$, $p = 0.03$; table 3), i.e. crickets that were heavier were larger and produced more sperm. The phenotypic correlations were less strongly positive than genetic correlations. Phenotypic correlations represented downward attenuated proxies of genetic correlations because within-individual residual correlations were weaker than genetic correlations (equations (1.1) and (1.2)).

4. Discussion

We investigated genetic and environmental correlations between pre- and post-mating traits to estimate the relative importance of trade-offs versus variation in male quality as drivers of phenotypic correlations between sexual traits in the field cricket. Our results provide support for both the male quality hypothesis (pre- and post-mating traits were positively integrated at the genetic levels) and the trade-offs hypothesis (the sign of within-individual changes in pre-mating traits was the opposite to that of post-mating traits with respect to testing order). Hence, by adopting a variance partitioning approach, we were able to reveal that multiple mechanisms (genetic and environmental) acted simultaneously in shaping phenotypic correlations between pre- and post-mating traits.

We found positive genetic correlations between traits encompassing pre- (male body size and aggressiveness) and post-mating selection (ejaculate size), a finding compatible with the hypothesis that the expression of sexual traits reflects male genetic quality. Sexual traits are conspicuous and energetically demanding and, therefore, only males of higher breeding values for fitness are able to bear their production and maintenance costs [50–52]. These males may not only allocate resources to pre-mating traits for accessing females but also efficiently allocate to post-mating traits, providing ejaculates most successful in fertilizations [10,23,53]. Eventually, traits such as body size and aggressiveness may signal male quality in both pre- (e.g. control of larger territories) and post-mating competitiveness (e.g. sperm competition). A recent meta-analysis indeed points to a tendency for pre-mating traits to be indicators of ejaculate quality (i.e. though close to zero, the association is positive) [2]. Hence, genotypes that can express and maintain costly sexual traits can become targets of female preference, as predicted by 'good genes' (pre-mating) and/or 'good sperm' (post-mating) models of mate choice [54,55]. In the southern field cricket larger and aggressive males have higher chances of winning male–male contests [56], gain access to breeding resources [31] and are preferred by females [32]. Ejaculate size (sperm number) is instead generally the main predictor of male fertilization success [57]. Thus, through mate choice, females can potentially derive indirect benefits by producing sons most successful in pre- and post-mating competition [58,59], reinforcing the positive genetic correlations between pre- and post-mating traits.

Variation in body mass can be genetic but also result from non-genetic (i.e. environmental) effects when some males have much more resources available to invest in multiple costly traits. However, our experimental manipulation of early-life environment did not support this idea. Whereas studies showed effects of nymph density on post-mating traits [60–63], our manipulation of rearing conditions revealed that males did not adjust their reproductive investment in response to competitive environments experienced during development. Possibly, nymphal densities represent poor predictors for future reproductive environments due to lack of sexual signals deriving from adults. Allocation shifts in crickets during development have been described in response to adult male calls (singing), most likely represent stronger indicators of intra-sexual competition [39,64]. Cricket aggression changes rapidly in response to recent social experiences [65,66] and ejaculate traits also depend on fighting [40], suggesting that their expressions are strongly determined by social interactions during adulthood rather than nymphal life.

Whereas associations between pre- and post-mating traits were positive at the genetic level, over repeated trials (testing order) positive changes in one trait were matched with negative changes in another: males decreased their aggression but increased production of sperm over repeated assays, indicating a within-individual trade-off in resource allocation between pre- and post-mating traits over the course of an individual's lifetime. Our previous studies also show that males of the southern field cricket decrease their aggressiveness over repeated trials [41,43]. The concurrent increase in sperm production, while decreasing aggressiveness, may be a consequence of enhanced sperm competition perceived through the repeated experiences of intra-sexual contests over time. This would explain why within-individual investments in pre- and post-mating traits changed with testing order in opposite directions, with males maximizing reproductive success by enhancing fertilization abilities (ejaculate

size) rather than allocating to winning fights (aggressiveness) over days of adult life [14,57]. These findings are unlikely to be driven by ageing, as we would have expected positive trait correlations (i.e. age-dependent decline in both traits). Within-individual correlations not attributable to testing order (i.e. true residual within-individual correlations) were close to zero. Such a finding was not unexpected as measurement error correlations (that aggregate at this statistical level [21]) were likely zero due to our study design. Interestingly, we did not find within-individual correlations among days that were not attributable to predictable changes with testing order, indicating the presence of gradual rather than rapid day-to-day changes in resource availability causing within-individual trade-offs.

Taken together, our results unambiguously demonstrate that the direction of correlations differed between hierarchical levels (phenotypic, genetic, permanent environmental and residual levels), implying that multiple biological mechanisms acted simultaneously. Such level-specific correlations and their effects on phenotypic correlations are increasingly reported, though primarily in behavioural and physiological research [18,67], as recently shown in a meta-analysis focusing on correlations between morphology, behaviour and physiology [67]. By contrast, studies of sexual selection estimating genetic correlations between pre- and post-mating traits have often not reported correlations at the within-individual level. Among these, quantitative genetic studies have reported negative genetic correlations between number of trills in courtship song and sperm viability in the field cricket *Teleogryllus oceanicus* [26], the iridescent area and sperm viability in the guppy *Poecilia reticulata* [27], attractiveness and size of salivary mass (a trait predicting fertilization success) in the scorpionfly *Panorpa eorgia* [28]. Other studies have instead reported positive genetic correlations between male body condition and testis weight in the dung beetle *Onthophagus taurus* [23], ejaculate size and body size (or sexual display) in the houbara bustard *Chlamydotis undulate undulata* [24]). Altogether, the literature implies that genetic correlation patterns attributable to trade-offs characterize some studies while genetic correlation patterns attributable to variation in male quality characterize other studies, demonstrating that among-study variation in phenotypic correlations between pre- and post-copulatory traits stems in part from population and species differences in genetic architecture.

Future research may also benefit from discriminating between types of reproductive traits (e.g. morphological, physiological or behavioural traits) for formulating testable predictions on trade-offs and quality effects at each hierarchical level of correlation, as some traits may become fixed at adulthood (e.g. weapons) while others are repeatedly expressed (e.g. behaviours). According to the type of trait, resources available for partitioning among multiple reproductive traits might either accumulate over long time periods (resulting in permanent environmental correlations) or not (resulting in within-individual correlations). For example, Somjee *et al*. [68] found a negative phenotypic correlation between morphological weaponry (hind femur size) and testes size allowing for increased sperm production in heliconia bug (*Leptoscelis tricolor*). Given the rearing environment (host plant) effects on resource allocation trade-offs [68], trade-offs may potentially exist at the developmental level, but they are unlikely to exist within-individuals as weaponry traits become fixed in adulthood. In our study, we were instead able to report within-individual correlations primarily because we focused on repeatedly expressed traits, such as aggressiveness and sperm production. Various simulations papers exist that provide recommendations on the optimal sampling designs that future studies may consult when planning to study level-specific correlations between pre- and post-reproductive traits [21,69].

The direction of phenotypic correlations between pre- and post-mating traits often differs between studies [2,5]. Variation in heritabilities of traits may represent one of the possible explanations. Equation (1) indeed implies that the relative contributions of genetic correlations and within-individual correlations depend on the magnitude of (geometric mean) heritabilities of traits. As heritabilities of correlated traits increase, the contribution of genetic correlations to phenotypic correlations increases while the contribution of within-individual correlations decreases in proportion [15]. In our study, heritabilities of aggression and sperm number were low due to large within-individual variability. In other words, the contribution of correlations non-genetic origin to the overall phenotypic correlation was relatively strong. As correlations of non-genetic origin were close to zero, phenotypic correlations represented downward attenuated estimates of genetic correlations, though qualitatively matching in sign. This finding underlines the importance of variance partitioning approaches when research aims to provide unbiased estimates of correlations at specific hierarchical levels [17]. Our study thereby demonstrates that comparisons of trait heritabilities and level-specific correlations should thus offer a powerful tool for explaining the lack of synthesis among studies of pre- and post-copulatory trait integration.

5. Conclusion

This paper provides empirical evidence that genetic variation in male quality drives positive phenotypic correlations between pre- and post-mating traits in southern field crickets, and that trade-offs drive negative trait correlations within individuals over their lifetime, while permanent environmental and residual within-individual correlations were, finally, zero. The existence of level-specific correlations implies that multiple mechanisms acted simultaneously, and variance partitioning is required to further our insight into the biological mechanisms shaping correlations between pre- and post-mating traits. Doing so may help explain why previous studies reported mixed results, thereby highlighting the importance of identifying multilevel variation in phenotypic traits and their correlations when studying integrated phenotypes.

Data accessibility. Data are available from the Dryad Digital Repository at: https://dx.doi.org/10.5061/dryad.34jf146 [70].

Authors' contributions. C.H. and C.T. conceived the study and collected data, C.H. carried out statistical analyses; all authors substantially contributed to drafting the manuscript, and gave final approval for publication.

Competing interests. We have no competing interests.

Funding. C.H. was funded by a Marie Curie Incoming International Fellowship (FP7-MC-IIF, 624672) and Basic Science Research Program through the National Research Foundation of Korea (NRF-2017R1A6A3A04002489), and C.T. by the 'BioNa Junior Scientist Award of the LMU'.

Acknowledgements. We thank Francesca Santostefano for providing crickets, Jessica Bacon, Jacob Ulcik and Ana-María Bastidas Urrutia for assistance in collecting data, Yvonne Cämmerer, Ekaterina Morozova, Bettina Rinjes and Franziska Wenzel for assistance in raising and breeding crickets, Herwig Stibor for access to the CASY.

References

1. Birkhead TR, Pizzari T. 2002 Postcopulatory sexual selection. *Nat. Rev. Genet.* **3**, 262–273. (doi:10.1038/nrg774)
2. Mautz BS, Møller AP, Jennions MD. 2013 Do male secondary sexual characters signal ejaculate quality? A meta-analysis. *Biol. Rev.* **88**, 669–682. (doi:10.1111/brv.12022)
3. Lupold S, Tomkins JL, Simmons LW, Fitzpatrick JL. 2014 Female monopolization mediates the relationship between pre- and postcopulatory sexual traits. *Nat. Commun.* **5**, 3184. (doi:10.1038/ncomms4184)
4. Evans JP, Garcia-Gonzalez F. 2016 The total opportunity for sexual selection and the integration of pre-and post-mating episodes of sexual selection in a complex world. *J. Evol. Biol.* **29**, 2338–2361. (doi:10.1111/jeb.12960)
5. Simmons LW, Lüpold S, Fitzpatrick JL. 2017 Evolutionary trade-off between secondary sexual traits and ejaculates. *Trends Ecol. Evol.* **32**, 964–976. (doi:10.1016/j.tree.2017.09.011)
6. Lande R, Arnold SJ. 1983 The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226. (doi:10.1111/j.1558-5646.1983.tb00236.x)
7. Lynch M, Walsh B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA: Sinauer.
8. Stearns SC. 1992 *The evolution of life histories*. Oxford, UK: Oxford University Press.
9. Roff DA. 2002 Life history evolution. *Recherche* **67**, 2.
10. Rahman MM, Kelley JL, Evans JP. 2013 Condition-dependent expression of pre- and postcopulatory sexual traits in guppies. *Ecol. Evol.* **3**, 2197–2213. (doi:10.1002/ece3.632)
11. Sheldon BC. 1994 Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. *Proc. R. Soc. B* **257**, 25–30. (doi:10.1098/rspb.1994.0089)
12. Fitzpatrick JL, Almbro M, Gonzalez-Voyer A, Kolm N, Simmons LW. 2012 Male contest competition and the coevolution of weaponry and testes in pinnipeds. *Evolution* **66**, 3595–3604. (doi:10.1111/j.1558-5646.2012.01713.x)
13. Emlen DJ. 2001 Costs and the diversification of exaggerated animal structures. *Science* **291**, 1534–1536. (doi:10.1126/science.1056607)
14. Parker GA, Lessells CM, Simmons LW. 2013 Sperm competition games: a general model for precopulatory male–male competition. *Evolution* **67**, 95–109. (doi:10.1111/j.1558-5646.2012.01741.x)
15. Roff DA. 1997 *Evolutionary quantitative genetics*. New York, NY: Chapman and Hall.
16. Hadfield JD, Nutall A, Osorio D, Owens IPF. 2007 Testing the phenotypic gambit: phenotypic, genetic and environmental correlations of colour. *J. Evol. Biol.* **20**, 549–557. (doi:10.1111/j.1420-9101.2006.01262.x)
17. Cheverud JM. 1988 A comparison of genetic and phenotypic correlations. *Evolution* **42**, 958–968. (doi:10.1111/j.1558-5646.1988.tb02514.x)
18. Dochtermann NA. 2011 Testing Cheverud's conjecture for behavioral correlations and behavioral syndromes. *Evolution* **65**, 1814–1820. (doi:10.1111/j.1558-5646.2011.01264.x)
19. Van Noordwijk AJ, de Jong G. 1986 Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**, 137–142. (doi:10.1086/284547)
20. Reznick D, Nunney L, Tessier A. 2000 Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol. Evol.* **15**, 421–425. (doi:10.1016/S0169-5347(00)01941-8)
21. Dingemanse NJ, Dochtermann NA. 2013 Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J. Anim. Ecol.* **82**, 39–54. (doi:10.1111/1365-2656.12013)
22. Nicolaus M, Piault R, Ubels R, Tinbergen JM, Dingemanse NJ. 2016 The correlation between coloration and exploration behaviour varies across hierarchical levels in a wild passerine bird. *J. Evol. Biol.* **29**, 1780–1792. (doi:10.1111/jeb.12907)
23. Simmons LW, Kotiaho JS. 2002 Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competition traits. *Evolution* **56**, 1622–1631. (doi:10.1111/j.0014-3820.2002.tb01474.x)
24. Chargé R, Teplitsky C, Hingrat Y, Saint Jalme M, Lacroix F, Sorci G. 2013 Quantitative genetics of sexual display, ejaculate quality and size in a lekking species. *J. Anim. Ecol.* **82**, 399–407. (doi:10.1111/1365-2656.12023)
25. Evans JP, Rahman MM, Gasparini C. 2015 Genotype-by-environment interactions underlie the expression of pre- and postcopulatory sexually selected traits in guppies. *J. Evol. Biol.* **28**, 959–972. (doi:10.1111/jeb.12627)
26. Simmons LW, Tinghitella RM, Zuk M. 2010 Quantitative genetic variation in courtship song and its covariation with immune function and sperm quality in the field cricket *Teleogryllus oceanicus*. *Behav. Ecol.* **21**, 1330–1336. (doi:10.1093/beheco/arq154)
27. Evans JP. 2010 Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. *Proc. R. Soc. B* **277**, 3195–3201. (doi:10.1098/rspb.2010.0826)
28. Engqvist L. 2011 Male attractiveness is negatively genetically associated with investment in copulations. *Behav. Ecol.* **22**, 345–349. (doi:10.1093/beheco/arq211)
29. Wilson AJ, Reale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB, Nussey DH. 2010 An ecologist's guide to the animal model. *J. Anim. Ecol.* **79**, 13–26. (doi:10.1111/j.1365-2656.2009.01639.x)
30. Adamo SA, Hoy RR. 1995 Agonistic behaviour in male and female field crickets, *Gryllus bimaculatus*, and how behavioural context influences its expression. *Anim. Behav.* **49**, 1491–1501. (doi:10.1016/0003-3472(95)90070-5)
31. Simmons LW. 1986 Inter-male competition and mating success in the field cricket, *Gryllus bimaculatus* (de Geer). *Anim. Behav.* **34**, 567–579. (doi:10.1016/S0003-3472(86)80126-9)
32. Simmons LW. 1986 Female choice in the field cricket *Gryllus bimaculatus* (De Geer). *Anim. Behav.* **34**, 1463–1470. (doi:10.1016/S0003-3472(86)80217-2)
33. Bretman A, Tregenza T. 2005 Measuring polyandry in wild populations: a case study using promiscuous crickets. *Mol. Ecol.* **14**, 2169–2179. (doi:10.1111/j.1365-294X.2005.02556.x)
34. Rodríguez-Muñoz R, Bretman A, Slate J, Walling CA, Tregenza T. 2010 Natural and sexual selection in a wild insect population. *Science* **328**, 1269–1272. (doi:10.1126/science.1188102)
35. Simmons LW, Denholm A, Jackson C, Levy E, Madon E. 2007 Male crickets adjust ejaculate quality with both risk and intensity of sperm competition. *Biol. Lett.* **3**, 520–522. (doi:10.1098/rsbl.2007.0328)
36. Gage AR, Barnard CJ. 1996 Male crickets increase sperm number in relation to competition and female size. *Behav. Ecol. Sociobiol.* **38**, 349–353. (doi:10.1007/s002650050251)
37. Gray B, Simmons LW. 2013 Acoustic cues alter perceived sperm competition risk in the field cricket *Teleogryllus oceanicus*. *Behav. Ecol.* **24**, 982–986. (doi:10.1093/beheco/art009)
38. Klaus SP, Fitzsimmons LP, Pitcher TE, Bertram SM. 2011 Song and sperm in crickets: a trade-off between pre- and post-copulatory traits or phenotype-linked fertility? *Ethology* **117**, 154–162. (doi:10.1111/j.1439-0310.2010.01857.x)
39. Bailey NW, Gray B, Zuk M. 2010 Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets. *Curr. Biol.* **20**, 845–849. (doi:10.1016/j.cub.2010.02.063)
40. Tuni C, Perdigón Ferreira J, Fritz Y, Munoz Meneses A, Gasparini C. 2016 Impaired sperm quality, delayed mating but no costs for offspring fitness in crickets winning a fight. *J. Evol. Biol.* **29**, 1643–1647. (doi:10.1111/jeb.12888)
41. Santostefano F, Wilson AJ, Araya-Ajoy YG, Dingemanse NJ. 2016 Interacting with the enemy: indirect effects of personality on conspecific

aggression in crickets. *Behav. Ecol.* **27**, 1235–1246. (doi:10.1093/beheco/arw037)

42. Han CS, Dingemanse NJ. 2017 You are what you eat: diet shapes body composition, personality and behavioural stability. *BMC Evol. Biol.* **17**, 8. (doi:10.1186/s12862-016-0852-4)
43. Han CS, Dingemanse NJ. 2017 Sex-dependent expression of behavioural genetic architectures and the evolution of sexual dimorphism. *Proc. R. Soc. B* **284**, 20171658. (doi:10.1098/rspb.2017.1658)
44. Santostefano F, Wilson AJ, Niemelä PT, Dingemanse NJ. 2017 Indirect genetic effects: a key component of the genetic architecture of behaviour. *Sci. Rep.* **7**, 10235. (doi:10.1038/s41598-017-08258-6)
45. Gasparini C, Lu C, Dingemanse NJ, Tuni C. 2017 Paternal-effects in a terrestrial ectotherm are temperature dependent but no evidence for adaptive effects. *Funct. Ecol.* **32**, 1011–1021. (doi:10.1111/1365-2435.13022)
46. Kruuk LEB. 2004 Estimating genetic parameters in natural populations using the 'animal model'. *Philos. Trans. R. Soc. Lond. B* **359**, 873–890. (doi:10.1098/rstb.2003.1437)
47. Santostefano F, Wilson AJ, Niemelä PT, Dingemanse NJ. 2017 Behavioural mediators of genetic life-history trade-offs: a test of the pace-of-life syndrome hypothesis in field crickets. *Proc. R. Soc. B* **284**, 20171567. (doi:10.1098/rspb.2017.1567)
48. Self SG, Liang K-Y. 1987 Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. *J. Am. Stat. Assoc.* **82**, 605–610. (doi:10.1080/01621459.1987.10478472)
49. Visscher PM. 2006 A note on the asymptotic distribution of likelihood ratio tests to test variance components. *Twin Res. Hum. Genet.* **9**, 490–495. (doi:10.1375/twin.9.4.490)
50. Zahavi A. 1975 Mate selection—a selection for a handicap. *J. Theor. Biol.* **53**, 205–214. (doi:10.1016/0022-5193(75)90111-3)
51. Grafen A. 1990 Biological signals as handicaps. *J. Theor. Biol* **144**, 517–546. (doi:10.1016/S0022-5193(05)80088-8)
52. Cotton S, Fowler K, Pomiankowski A. 2004 Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. R. Soc. B* **271**, 771–783. (doi:10.1098/rspb.2004.2688)
53. Perry JC, Rowe L. 2010 Condition-dependent ejaculate size and composition in a ladybird beetle. *Proc. R. Soc. B* **277**, 3639–3647. (doi:10.1098/rspb.2010.0810)
54. Kokko H, Brooks R, Jennions MD, Morley J. 2003 The evolution of mate choice and mating biases. *Proc. R. Soc. B* **270**, 653–664. (doi:10.1098/rspb.2002.2235)
55. Andersson M, Simmons LW. 2006 Sexual selection and mate choice. *Trends Ecol. Evol.* **21**, 296–302. (doi:10.1016/j.tree.2006.03.015)
56. Hofmann HA, Schildberger K. 2001 Assessment of strength and willingness to fight during aggressive encounters in crickets. *Anim. Behav.* **62**, 337–348. (doi:10.1006/anbe.2001.1746)
57. Parker GA, Pizzari T. 2010 Sperm competition and ejaculate economics. *Biol. Rev.* **85**, 897–934. (doi:10.1086/656840)
58. Wedell N, Tregenza T. 1999 Successful fathers sire successful sons. *Evolution* **53**, 620–625. (doi:10.1111/j.1558-5646.1999.tb03797.x)
59. Head ML, Hunt J, Jennions MD, Brooks R, Partridge L. 2005 The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol.* **3**, e33. (doi:10.1371/journal.pbio.0030033)
60. Gage MJG. 1995 Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. B* **261**, 25–30. (doi:10.1098/rspb.1995.0112)
61. Stockley P, Seal NJ. 2001 Plasticity in reproductive effort of male dung flies (*Scatophaga stercoraria*) as a response to larval density. *Funct. Ecol.* **15**, 96–102. (doi:10.1046/j.1365-2435.2001.00496.x)
62. McNamara KB, Elgar MA, Jones TM. 2010 Adult responses to larval population size in the almond moth, *Cadra cautella*. *Ethology* **116**, 39–46. (doi:10.1111/j.1439-0310.2009.01714.x)
63. He Y, Miyata T. 1997 Variations in sperm number in relation to larval crowding and spermatophore size in the armyworm, *Pseudaletia separata*. *Ecol. Entomol.* **22**, 41–46. (doi:10.1046/j.1365-2311.1997.00030.x)
64. Kasumovic MM, Hall MD, Try H, Brooks RC. 2011 The importance of listening: juvenile allocation shifts in response to acoustic cues of the social environment. *J. Evol. Biol.* **24**, 1325–1334. (doi:10.1111/j.1420-9101.2011.02267.x)
65. Hofmann HA, Stevenson PA. 2000 Flight restores fight in crickets. *Nature* **403**, 613. (doi:10.1038/35001137)
66. Killian KA, Allen JR. 2008 Mating resets male cricket aggression. *J. Insect Behav.* **21**, 535–548. (doi:10.1007/s10905-008-9148-x)
67. Niemelä PT, Dingemanse NJ. 2018 Meta-analysis reveals weak associations between intrinsic state and personality. *Proc. R. Soc. B* **285**, 20172823. (doi:10.1098/rspb.2017.2823)
68. Somjee U, Allen PE, Miller CW. 2015 Different environments lead to a reversal in the expression of weapons and testes in the heliconia bug, *Leptoscelis tricolor* (Hemiptera: Coreidae). *Biol. J. Linn. Soc.* **115**, 802–809. (doi:10.1111/bij.12544)
69. Morrissey MB, Wilson AJ, Pemberton JM, Ferguson MM. 2007 A framework for power and sensitivity analyses for quantitative genetic studies of natural populations, and case studies in Soay sheep (*Ovis aries*). *J. Evol. Biol.* **20**, 2309–2321. (doi:10.1111/j.1420-9101.2007.01412.x)
70. Tuni C, Han CS, Dingemanse NJ. 2018 Data from: Multiple biological mechanisms result in correlations between pre- and post-mating traits that differ among versus within individuals and genotypes. Dryad Digital Repository. (https://doi.org/10.5061/dryad.34jf146)