Short title: *Rensch's rule in Tribolium*
Title for authors: *O. Y. Martin et al.*

Correspondence: Oliver Y. Martin, ETH Zürich, Experimental Ecology, Institute of Integrative Biology, D-USYS, Universitätsstrasse 16, CH-8092 Zürich, Switzerland
Fax: +41 44 632 12 71, E-mail: oliver.martin@env.ethz.ch

Lack of support for Rensch's rule in an intraspecific test using red flour beetle (*Tribolium castaneum*) populations

Oliver Y. Martin[1,2], Łukasz Michalczyk[1,3], Anna L. Millard[1], Brent C. Emerson[1,4] and Matthew J. G. Gage[1]

[1]*School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom,* [2]*ETH Zürich, Experimental Ecology, Institute of Integrative Biology, D-USYS, Universitätsstrasse 16, CH-8092 Zürich, Switzerland,* [3]*Department of Entomology, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland, and* [4]*Island Ecology and Evolution Research Group (IPNA-CSIC), C/Astrofísico Francisco Sánchez 3, 38206 La Laguna, Tenerife, Canary Islands, Spain*

This is an Accepted Article that has been peer-reviewed and approved for publication in the Insect Science but has yet to undergo copy-editing and proof correction. Please cite this article as doi: 10.1111/1744-7917.12272.

Abstract Rensch's rule proposes a universal allometric scaling phenomenon across species where sexual size dimorphism (SSD) has evolved: in taxa with male-biased dimorphism, degree of SSD should increase with overall body size, and in taxa with female-biased dimorphism, degree of SSD should decrease with increasing average body size. Rensch's rule appears to hold widely across taxa where SSD is male-biased, but not consistently when SSD is female-biased. Furthermore, studies addressing this question within species are rare, so it remains unclear whether this rule applies at the intraspecific level. We assess body size and SSD within *Tribolium castaneum* (Herbst, 1797), a species where females are larger than males, using 21 populations derived from separate locations across the world, and maintained in isolated laboratory culture for at least 20 years. Body size, and hence SSD patterns, are highly susceptible to variations in temperature, diet quality and other environmental factors. Crucially, here we nullify interference of such confounds as all populations were maintained under identical conditions (similar densities, standard diet and exposed to identical temperature, relative humidity and photoperiod). We measured thirty beetles of each sex for all populations, and found body size variation across populations, and (as expected) female-biased SSD in all populations. We test whether Rensch's rule holds for our populations, but find isometry, i.e. no allometry for SSD. Our results thus show that Rensch's rule does not hold across populations within this species. Our intraspecific test matches previous interspecific studies showing that Rensch's rule fails in species with female-biased SSD.

Key words allometry, body size, Coleoptera, sexual selection, sexual size dimorphism, Tenebrionidae

Introduction

Differences in male and female body size within a given species are ubiquitous in the animal kingdom. Sexual size dimorphism (SSD) describes how the body sizes of the sexes relate to each other, and a range of selective pressures have so far been invoked to explain this widespread phenomenon (Fairbairn, 1997; Blanckenhorn, 2000, 2005). The diverse selective pressures that act on female and male body size can relate to sexual selection, as well as natural selection or fecundity selection. For instance, male-male competition over territories or resources, as well as female choice for larger-bodied males, could drive male-biased SSD (Darwin, 1871). There could also be selection for smaller males, if these are more mobile or agile, and this could drive female-biased SSD (Andersson & Norberg, 1981; Zamudio, 1998).

Whereas some animal groups such as mammals generally show a pattern of male-biased SSD (i.e. males are the larger sex), in other groups such as spiders or fish, SSD tends to be female-biased (i.e. females are the larger sex). The degree to which male and female body sizes differ is highly variable, both between and within species. Size differences between the sexes can range from barely perceptible to extremely pronounced. For example, stark contrasts are found in some spider species, where males are less than 10% of the size of females (see Elgar, 1991). Despite recognition that SSD is so widespread (Stillwell *et al*., 2010), the major underlying mechanisms responsible generally remain elusive (Blanckenhorn, 2000, 2005). Particular drivers, such as sexual or natural selection (Blanckenhorn, 2000; Lindenfors *et al*., 2007; Allen *et al*., 2011) may conceivably favor different optimal sizes for males and females, yet matters are complicated by genetic correlations of shared traits (Lande, 1980).

More rapid divergence over evolutionary time is expected in male *versus* female body size, due to the latter being more constrained, and the resulting interspecific pattern in SSD variation is described by Rensch's rule (Rensch, 1950; Fairbairn, 1997, Blanckenhorn *et al.*, 2007a, b). Specifically, the rule predicts that with increasing species body sizes, male-biased

SSD will increase and female-biased SSD will decrease. Rensch's rule appears to be generally applicable in taxa where SSD is male-biased (e.g. Polak & Frynta, 2010), but not consistently when the pattern is one of female-biased SSD (Blanckenhorn *et al*., 2007a, Webb & Freckleton, 2007, Cueva del Castillo & Fairbairn, 2012; Liao *et al*., 2013; but see Stuart-Fox, 2009). Considering that so many animal species, e.g. ca. 88% of insects (Stillwell *et al*., 2010), exhibit female-biased SSD, this represents a severe restriction on the general usefulness of this 'rule'. Considering the importance of insects in terms of biodiversity and biomass, further evidence from this important group would be welcome (Blanckenhorn *et al*., 2007a). In addition to further information from female-biased dimorphic taxa, informative tests of Rensch's rule can be made using intraspecific, interpopulation SSD and body size variation (Blanckenhorn *et al*., 2007a). Based on the few within-species studies available, evidence is so far inconclusive. Whereas Fairbairn and Preziosi (1994) find support for Rensch's rule in water strider populations, studies on other insect species (dung flies: Kraushaar & Blanckenhorn, 2002; bushcrickets: Eweleit & Reinhold, 2014) or humans (Gustafsson & Lindenfors, 2004) do not. Similarly, in a study focusing on SSD and Rensch's rule in blennies, Rensch's rule was only confirmed in two of the three species assessed (Lengkeek *et al*., 2008). So, overall, more studies focusing on intraspecific tests would be useful to determine whether Rensch's rule can be generally applied.

The red flour beetle *Tribolium castaneum* (Herbst, 1797) shows a female-biased SSD, and has been an informative model system for the study of various aspects of sexual selection and conflict (see Michalczyk *et al*., 2010, 2011b; Sbilordo *et al*., 2011; Grazer & Martin, 2012), including targeted experimental evolution studies (Michalczyk *et al*., 2011a, Kerstes *et al*., 2013; Demont *et al*., 2014). Due to intense interest in this coleopteran as an important stored food pest with global distribution, a large range of laboratory stocks derived from widespread locations have been maintained in laboratory culture. Extensive genetic resources are available, including a fully sequenced and annotated genome (*Tribolium* Genome Sequencing Consortium, 2008). *T. castaneum* has a long history of service as a model system of choice in various contexts, generating extensive knowledge of general species biology (see Sokoloff, 1972, 1974), including directly relevant aspects such as body size evolution (Conner & Via, 1992). More recently, the evolution of SSD in this species has been investigated using an artificial selection approach (Tigreros & Lewis, 2011). Beetles were subjected to sexually antagonistic selection, achieved by selecting on body size in opposite directions in males and females. The authors concluded that sex-specific larval development may drive SSD in this system (Tigreros & Lewis, 2011).

Here we use a range of wild type *T. castaneum* populations (representing a degree of variation in body size) derived from separate geographic locations and maintained under standard lab conditions for several years. Body size and hence SSD patterns are known to be generally highly susceptible to developmental influences from temperature (*Temperature-Size Rule*: Atkinson, 1994; Angiletta, 2009). Furthermore, humidity, photoperiod, diet quality or other environmental influences can also have strong impacts (see e.g. Litzgus & Smith, 2010; Stillwell *et al*., 2010; Laiolo *et al.* 2013; Çağlar *et al*., 2014). This is particularly important in this context, as it has been predicted that females and males may respond to environmental conditions in different ways, generating variance in SSD across environments (see ‘differential plasticity hypothesis’: Fairbairn, 2005; Teder & Tammaru, 2005). Here we eliminate interference of many potentially confounding factors as our beetle stocks were maintained under standardised environmental conditions for long periods of time (at least 20 years). In our lab, beetle populations were housed before measurement in the same climate chamber at similar densities, exposed to the same constant temperature, relative humidity conditions and photoperiod as well as given the same standard diet. This model system therefore presents an excellent opportunity for a direct intraspecific test of Rensch’s rule where significant body size variation exists, despite different populations being isolated and maintained under uniform environmental conditions for more than 20 years.

Materials and methods

Sample populations

Twenty wild type *T. castaneum* populations of independent origin were assessed in this study (Table 1 for details). In addition, we measured female and male beetles of the Reindeer (Rd) stock. Rd beetles have a dominant mutation generating antennae of markedly different shape compared with wild type. As Rd can easily be identified by eye, they have proved very useful as a phenotypic marker strain in sperm competition studies (Michalczyk *et al*., 2010, 2011a; Sbilordo *et al*., 2011; Sbilordo & Martin, 2014). All beetle populations were derived from stocks obtained from Richard Beeman at USDA and held in our laboratory for over a year before measurement, where they were reared on medium containing 9 weight units of white plain organic flour and 1 weight unit of powdered organic brewer's yeast provided *ad libitum*. The use of standard food is important as diet quality can have profound effects on developmental time and hence body size and weight of offspring in *Tribolium* (Sokoloff, 1972, 1974; Sverdlov & Wool, 1973; Via & Conner, 1995). Stocks were maintained in the same climate chamber at 30° C and 65% relative humidity with a 16h light : 8h dark cycle. Again, maintaining beetles in one climate chamber under standard conditions is critical, as factors such as temperature and relative humidity (Park & Frank, 1948; Howe, 1962; Grazer & Martin, 2012) or food quality (Fedina & Lewis, 2006; Fedina, 2007; Sbilordo *et al*., 2011) affect various reproductive traits and body size in this species (see also Sokoloff, 1972, 1974).

Beetle stocks were kept in 12 × 12 × 12 cm plastic boxes covered with lids incorporating 7 × 7 cm windows of fine metal mesh. At least two boxes for each strain were maintained in culture, and populations were maintained at *ca.* 500 adult beetles per box to standardize population density and avoid confounding effects associated with differences in local sexual selection intensity (e.g. Gavrilets, 2000; Martin & Hosken, 2003, 2004). Each box contained *ca.* 250 g of rearing medium (see above). The amount of flour provided is known to affect the number and size of offspring (e.g. Campbell & Runnion, 2003). The food medium was therefore provided in abundance to counter possible impacts on adult body size due to differences in larval density and/or resulting larval competition.

Body size measurements and statistical procedures

The body sizes of thirty sexually mature beetles of each sex (all *ca.* 2 weeks old) were measured for each of 21 stocks of *T. castaneum*. Beetles were frozen alive at –18° C and elytra lengths measured under a stereomicroscope at 20× magnification with an eyepiece measuring graticule. Elytra length is routinely used as a measure of body size for this species (see e.g. Lewis & Austad, 1990), and was thus chosen to enable comparisons with previous work. Based on these measurements, one commonly used index of sexual size dimorphism (SSD) (Lovich & Gibbons, 1992), was calculated for each population in the study: SDI = [(*Female size/Male size*) – 1] × 1 if female is the larger sex (as is the case in *T. castaneum*) (for calculation of a range of other indices from this data, see e.g. Smith, 1999; Blanckenhorn *et al.*, 2006). The information on male and female body size and SDIs (Table 1) is supplemented with an allometric regression plot of male on female body size (Fig. 1).

Statistically, a pattern consistent with Rensch's rule would be evident as significant hypoallometry (SSD increases with decreasing body size), i.e. as *a* slope of $b < 1$ (Fairbairn & Preziosi, 1994). As random error is expected in both y and x (see e.g. Schulte-Hostedde *et al.*, 2005), it is more appropriate to estimate the slope via reduced major axis (RMA) regression (Sokal & Rohlf, 1995). To do this, we used an Excel spreadsheet designed for calculation of RMA and MA slopes that was supplied by Wolf Blanckenhorn (see Fairbairn, 2005, Blanckenhorn *et al*., 2007b). The slope was additionally estimated using the RMA software for JAVA 1.21 (see Bohonak & van der Linde, 2004). Overall, we investigated whether the slope showed a pattern of hypoallometry, and was hence consistent with Rensch's rule, via inspection of overlap of the 95% confidence intervals (see Fairbairn, 2005; Schulte-Hostedde *et al*., 2005).

Results

Intraspecific variation in SSD

An overview of mean female and male body sizes (elytra lengths) and SDIs for each population assessed is given in Table 1. In addition to mean body sizes we provide one index of SSD for each population, i.e. SDIs (Lovich & Gibbons, 1992). As expected, females are on average larger than males in all populations assessed (all points above the hatched line of isometry in Fig. 1).

Rensch's rule

We found that mean female and male body sizes were significantly correlated ($\log_{10}$ body sizes; linear regression: $R^2 = 0.882$, $F_{1,19} = 141.425$, $P < 0.0001$; Fig. 1). Using the Excel spreadsheet (see Blanckenhorn *et al*., 2007b, Fairbairn, 2005), we obtain a RMA slope of 1.022 for raw data (95% confidence limits of 0.859 and 1.221; Least Squares (LS) slope 0.961, SE 0.058, Major Axis (MA) slope 1.024, SE 0.038). For $\log_{10}$ body sizes, the RMA slope equals 0.983 (95% confidence limits of 0.823 and 1.172; LS slope 0.923, SE 0.055, MA slope 0.982, SE 0.037). Results were additionally confirmed using RMA software for JAVA 1.21 (see above). All regression analyses performed indicate that the slope is not significantly different from *b*=1 (i.e. a pattern of isometry, no allometry for SSD). Our results therefore do not fit Rensch's rule (i.e. significant hypoallometry).

Discussion

Here, we use *T. castaneum* wild type strains kept under identical standard conditions, but varying across populations by ~20% in average body size. This variation is likely due to genetic differences, rather than the result of environmental influences as populations were maintained under exactly the same conditions. Using these populations, we are able to provide a targeted intraspecific test of Rensch's rule, and show that the rule does not hold for this female-biased size dimorphic species (i.e. we find a pattern of isometry, not one of hypoallometry). It is worth stressing that although there are numerous examples of Rensch's rule in groups where males represent the larger sex, many tests of the rule in taxa with exclusively female-biased SSD have failed to confirm the pattern (Abouheif & Fairbairn, 1997; Fairbairn, 1997; Tubaro & Bertelli, 2003, Blanckenhorn *et al*., 2007a, Webb & Freckleton, 2007, Cueva del Castillo & Fairbairn, 2012).

It is generally accepted that the evolution of male-biased SSD may be driven primarily by sexual selection (see Stilwell *et al*., 2010). In taxa showing male-biased SSD, Rensch's rule may generally hold more frequently due to the combination of sexual selection on larger male body size with weaker correlated selection on female size (Szekely *et al*., 2004). The evolution of female-biased SSD, in contrast, is still poorly understood, and possibly "driven by a more diverse and complex range of selective processes" (see Stuart-Fox, 2009). For example, larger female size could be due to sex role reversal (i.e. females compete for access to males), and this is common in birds with female-biased SSD (see Tubaro & Bertelli, 2003). Or, rather than selection for large females, there may also be selection for smaller males, if these are more agile, for instance (see Andersson & Norberg, 1981; Zamudio, 1998). Because of these diverse selective pressures, Rensch's rule may be expected to fit less consistently when taxa show female-biased SSD, as is the case here in *T. castaneum* populations studied. For *T. castaneum,* the interplay of selective pressures responsible for female-biased SSD and the isometric pattern found remains to be captured.

Comparative studies generally do not take into account potentially substantial seasonal or regional differences. Body size is very sensitive to variation in temperature or food quality, and this can also affect patterns of SSD via the degree of dimorphism (e.g. Hu *et al*., 2010). Indeed, body size of both sexes is known to be susceptible to variation in temperature and humidity in *Tribolium* (Sokoloff, 1974). Here we deliberately use different flour beetle populations, which vary in their average body sizes, but are maintained under standardized conditions on the same food and under the same temperature and humidity conditions. This allows us to avoid the known environmental effects on a range of traits in this species (e.g. Howe, 1962; Fedina & Lewis, 2006; Sbilordo *et al*., 2011; Grazer & Martin, 2012). Patterns of SSD documented here are far less prone to potentially confounding variation present in data from samples collected from different locations and at different times, or taken from heterogeneous sources from the literature, as would often be the case for comparative approaches.

It has been recognized that more work would be needed addressing population differences in SSD (Jannot & Kerans, 2003). Intraspecific studies remain rare, so our study provides a valuable test of Rensch's rule, which is relatively free of confounding variation from the environment or phylogenetic inertia. Furthermore, our system is ideally suited to addressing interpopulation variation because the separate stocks used have been maintained and undergone simultaneous culturing for many years. Because SSD is thought to be tightly associated with ecological conditions and life-history strategies, and hence closely linked with the phylogeny of study species, intraspecific testing provides an important complement to cross-species analyses (Brooks & McLennan, 1991).

In conclusion, we show that Rensch's rule does not hold across populations of the flour beetle *Tribolium castaneum*, a species where females are larger than males. In this respect, our intraspecific test matches previous interspecific studies showing that Rensch's rule fails in species with female-biased SSD (e.g. Blanckenhorn *et al*., 2007a; Webb & Freckleton, 2007; Guillermo-Ferreira *et al.*, 2014; Hirst & Kiørboe, 2015). Crucially, as we are able to limit common environmental sources of variation through the use of laboratory stocks kept under standard conditions, our results are likely to be particularly robust. Understanding the precise selective pressures responsible for the isometric pattern found here could be the subject of future study, building on the wealth of data already available concerning the action of sexual selection (and to a lesser extent, natural selection) in this species.

Acknowledgments

The authors thank Richard Beeman (USDA) for generously sending us the beetles used to start our lab stocks, and Wolf Blanckenhorn for kindly providing a copy of an Excel spreadsheet designed for calculation of RMA and MA slopes. The authors further thank NERC (Standard research grant to MJGG, BCE and OYM), Swiss National Science Foundation (postdoctoral fellowships and Ambizione grants to OYM), the University of East Anglia and ETH Zürich for support, and the anonymous reviewers for comments on the manuscript.

Disclosure

The authors declare that they have no conflict of interests.

References

Abouheif, E. and Fairbairn, D.J. (1997) A comparative analysis of allometry for sexual size dimorphism: Assessing Rensch's rule. *American Naturalist*, 149, 540–562.

Allen, C.E., Zwaan, B.J. and Brakefield, P.M. (2011) Evolution of sexual dimorphism in the Lepidoptera. *Annual Review of Entomology*, 56, 445–464.

Andersson, M. and Norberg, A. (1981) Evolution of reversed sexualsize dimorphism and role partitioning among predatorybirds, with a size scaling of flight performance. *Biological Journal of the Linnean Society*, 15, 105–130.

Angilletta, M.J. (2009) *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford, UK.

Atkinson, D. (1994) Temperature and organism size – A biological law for ectotherms. *Advances in Ecological Research*, 25, 1–58.

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology*, 75, 385–407.

Blanckenhorn, W.U. (2005) Behavioral causes and consequences of sexual size dimorphism. *Ethology*, 111, 977–1016.

Blanckenhorn, W.U., Meier, R. and Teder, T. (2007a) Rensch's rule in insects: Patterns among and within species. *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism* (eds. D.J. Fairbairn, W.U. Blanckenhorn, & T. Szekely), pp. 60–70. Oxford University Press, Oxford, UK.

Blanckenhorn, W.U., Stillwell, R.C., Young, K.A., Fox, C.W. and Ashton, K.G. (2006) When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? *Evolution*, 60, 2004–2011.

Blanckenhorn, W.U., Dixon, A.F.G., Fairbairn, D.J., Foellmer, M.W., Gibert, P., van der

Linde, K., Meier, R., Nylin, S., Pitnick, S., Schoff, C., Signorelli, M., Teder, T. and Wiklund, C. (2007b) Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? *American Naturalist*, 169, 245–257.

Bohonak, A.J. and van der Linde, K. (2004) RMA: Software for Reduced Major Axis regression, Java version. Website: http://www.kimvdlinde.com/professional/rma.html.

Brooks, D.R. and McLennan, D.A. (1991) *Phylogeny, Ecology, and Behaviour: A Research Program in Comparative Biology*. University of Chicago Press, Chicago, USA.

Çağlar, S.S., Çağaşan, K., Cem Kuyucu, A. and Sağlam, I.K. (2014) Humidity and seasonality drives body size patterns in males of the bush cricket *Isophya rizeensis* Sevgili, 2003 (Orthoptera: Tettigoniidae: Phaneropterinae). *Insect Science*, 21, 213–226.

Campbell, J.F. and Runnion, C. (2003) Patch exploitation by female red flour beetles, *Tribolium castaneum*. *Journal of Insect Science*, 3, 20.

Conner, J. and Via, S. (1992) Natural selection on body size in *Tribolium*: possible genetic constraints on adaptive evolution. *Heredity*, 69, 73–83.

Cueva del Castillo, R. and Fairbairn, D.J. (2012) Macroevolutionary patterns of bumblebee body size: detecting the interplay between natural and sexual selection. *Ecology and Evolution*, 2, 46–57.

Demont, M., Grazer, V.M., Michalczyk, Ł., Millard, A.L., Sbilordo, S.H., Emerson, B.C., Gage, M.J.G. and Martin, O.Y. (2013) Experimental removal of sexual selection reveals adaptations to polyandry in both sexes. *Evolutionary Biology*, 41, 62-70.

Elgar, M.A. (1991) Sexual cannibalism, size dimorphism and courtship behavior in orb-weaving spiders (Araneae). *Evolution*, 45, 444–448.

Eweleit, L. and Reinhold, K. (2014) Body size and elevation: do Bergmann's and Rensch's rule apply in the polytypic bushcricket *Poecilimon veluchianus*? *Ecological Entomology*, 39, 133–136.

Fairbairn, D.J. (1997) Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology, Evolution, and Systematics*, 28, 659–687.

Fairbairn, D.J. (2005) Allometry for sexual size dimorphism: testing two hypotheses for Rensch's rule in the water strider *Aquarius remigis*. *American Naturalist*, 166, S69–S84.

Fairbairn, D.J. and Preziosi, R.F. (1994) Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider *Aquarius remigis*. *American Naturalist*, 144, 101–118.

Fedina, T. (2007) Cryptic female choice during spermatophore transfer in *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Insect Physiology*, 53, 93–98.

Fedina, T. and Lewis, S.M. (2006) Proximal traits and mechanisms for biasing paternity in the red flour beetle *Tribolium castaneum. Behavioral Ecology and Sociobiology*, 60, 844–853.

Gavrilets, S. (2000) Rapid evolution of reproductive barriers driven by sexual conflict. *Nature*, 403, 886–889.

Grazer, V.M. and Martin, OY. (2012) Elevated temperature changes female costs and benefits of reproduction. *Evolutionary Ecology*, 26, 715–731.

Guillermo-Ferreira, R., Novaes, M.C., Lecci, L.S. and Bispo, P.C. (2014) Allometry for sexual size dimorphism in stoneflies sefies the Rensch's rule. *Neotropical Entomology*, 43, 172–175.

Gustafsson, A. and Lindenfors, P. (2004) Human size evolution: no evolutionary allometric relationship between male and female stature. *Journal of Human Evolution*, 47, 253–

266.

Hirst, A.G. and Kiørboe, T. (2015) Macroevolutionary patterns of sexual size dimorphism in copepods. *Proceedings of the Royal Society B: Biological Sciences*, 282, in press.

Howe, R.W. (1962) The effects of temperature and humidity on the oviposition rate of *Tribolium castaneum* (Hbst.) (Coleoptera, Tenebrionidae). *Bulletin of Entomological Research,* 53, 301–310.

Hu, Y., Xie, Y., Wang, C. and Lei, C. (2010) Variation in sexual size dimorphism among populations: testing the differential-plasticity hypothesis. *Entomologia Experimentalis et Applicata*, 137, 204–209.

Jannot, J.E. and Kerans, B.L. (2003) Body size, sexual size dimorphism, and Rensch's rule in adult hydropsychid caddisflies (Trichoptera: Hydropsychidae). *Canadian Journal of Zoology*, 81, 1956–1964.

Kerstes, N.A.G., Bérénos, C. and Martin, O.Y. (2013) Coevolving parasites and population size shape the evolution of mating behaviour. *BMC Evolutionary Biology*, 13, 29.

Kraushaar, U. and Blanckenhorn, W,U. (2002) Population variation in sexual selection and its effect on body size allometry in two species of flies with contrasting sexual size dimorphism. *Evolution*, 56, 307–321.

Laiolo, P., Illera, J.C. and Obeso, J.R. (2013) Local climate determines intra- and interspecific variation in sexual size dimorphism in mountain grasshopper communities. *Journal of Evolutionary Biology*, 26, 2171–2183.

Lande, R. (1980) Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution*, 34, 292–305.

Lengkeek,W., Didderen, K., Côté, I.M., van der Zee, E.M., Snoek, R.C. and Reynolds, J.D. (2008) Plasticity in sexual size dimorphism and Rensch's rule in Mediterranean blennies (Blenniidae). *Canadian Journal of Zoology*, 86, 1173–1176.

Lewis, S.M and Austad, S.N. (1990) Sources of intraspecific variation in sperm precedence in red flour beetles. *American Naturalist*, 135, 351–359.

Liao, W.B., Zeng, Y., Zhou, C.Q and Jehle, R. (2013) Sexual size dimorphism in anurans fails to obey Rensch's rule. *Frontiers in Zoology*, 10, 10.

Lindenfors, P., Gittleman, J.L. and Jones, K.E. (2007) Sexual size dimorphism in mammals. *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism* (eds. D.J. Fairbairn, W.U. Blanckenhorn, & T. Szekely), pp. 16–26. Oxford University Press, Oxford, UK.

Litzgus, J.D. and Smith, S.E. (2010) Geographic variation in sexual size dimorphism in painted turtles (*Chrysemys picta*). *Journal of Herpetology*, 44, 320–326.

Lovich, J.E. and Gibbons, J.W. (1992) A review of techniques for quantifying sexual size dimorphism. *Growth Development & Aging*, 56, 269–281.

Martin, O.Y. and Hosken, D.J. (2003) The evolution of reproductive isolation through sexual conflict. *Nature*, 423, 979–982.

Martin, O.Y. and Hosken, D.J. (2004) Reproductive consequences of population divergence through sexual conflict. *Current Biology*, 14, 906–910.

Michalczyk, Ł., Martin, O.Y., Millard, A.L., Emerson, B.C. and Gage, M.J.G. (2010) Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male *Tribolium castaneum*. *Proceedings of the Royal Society B: Biological Sciences*, 277, 3483–3491.

Michalczyk, Ł., Millard, A.L., Martin, O.Y., Lumley, A., Emerson, B.C. and Gage, M.J.G. (2011a) Experimental evolution exposes female and male responses to sexual selection and conflict in *Tribolium castaneum*. *Evolution*, 65, 713–724.

Michalczyk, Ł., Millard, A.L., Martin, O.Y., Lumley, A., Emerson, B.C., Chapman, T. and Gage, M.J.G. (2011b) Inbreeding promotes female promiscuity. *Science*, 333, 1739–

1742.

Park, T. and Frank, M.B. (1948) The fecundity and development of the flour beetles *Tribolium confusum* and *Tribolium castaneum* at three constant temperatures. *Ecology*, 29, 368–374.

Polak, J. and Frynta, D. (2010) Patterns of sexual size dimorphism in cattle breeds supports Rensch's rule. *Evolutionary Ecology*, 24, 1255–1256.

Rensch, B. (1950) Die Abhängigkeit der relativen Sexualdifferenz von der Körpergrösse. *Bonner Zoologische Beiträge*, 1, 58–69.

Sbilordo, S.H. and Martin, O.Y. (2014) Pre- and postcopulatory sexual selection act in concert to determine male reproductive success in *Tribolium castaneum*. *Biological Journal of the Linnean Society*, 112, 67–75.

Sbilordo, S.H., Grazer, V.M., Demont, M. and Martin, O.Y. (2011) Impacts of starvation on male reproductive success in *Tribolium castaneum*. *Evolutionary Ecology Research*, 13, 347–359.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.S. and Hickling, G.J. (2005) Restitution of mass-size residuals: validating body condition indices. *Ecology*, 86, 155–163.

Smith, R.J. (1999) Statistics of sexual size dimorphism. *Journal of Human Evolution*, 36, 423–459.

Sokal, R.R. and Rohlf, F.J. (1995) Biometry: the principles and practice of statistics in biological research. W. H. Freeman, New York, USA.

Sokoloff, A. (1972) The Biology of Tribolium with Special Emphasis on Genetic Aspects, Volume 1. Clarendon Press, Oxford, UK.

Sokoloff, A. (1974) The Biology of Tribolium with Special Emphasis on Genetic Aspects, Volume 2. Clarendon Press, Oxford, UK.

Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G. and Fox, C.W. (2010) Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annual Review of Entomology*, 55, 227–245.

Stuart-Fox, D. (2009) A test of Rensch's rule in dwarf chameleons (*Bradypodion* spp.), a group with female-biased sexual size dimorphism. *Evolutionary Ecology*, 23, 425–433.

Sverdlov, E. and Wool, D. (1973) The effect of rearing *Tribolium castaneum* in used culture medium on the developmental time of the next generation. *Entomologia Experimentalis et Applicata*, 16, 550–556.

Szekely, T., Freckleton, R.P. and Reynolds, J.D. (2004) Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *Proceedings of the National Academy of Sciences USA*, 101, 12224–12227.

Teder, T. and Tammaru, T. (2005) Sexual size dimorphism within species increases with body size in insects. *Oikos*, 108, 321–334.

Tigreros, N. and Lewis, S.M. (2011) Direct and correlated responses to artificial selection on sexual size dimorphism in the flour beetle, *Tribolium castaneum*. *Journal of Evolutionary Biology*, 24, 835–842.

Tribolium Genome Sequencing Consortium (2008) The genome of the model beetle and pest *Tribolium castaneum*. *Nature*, 452, 949–55.

Tubaro, P.L. and Bertelli, S. (2003) Female-biased sexual size dimorphism in tinamous: a comparative test fails to support Rensch's rule. *Biological Journal of the Linnean Society*, 80, 519–527.

Via, S. and Conner, J. (1995) Evolution in heterogeneous environments: genetic variability within and across different grains in *Tribolium castaneum*. *Heredity,* 74, 80–90.

Webb, T.J. and Freckleton, R.P. (2007) Only half right: species with female-biased sexual size dimorphism consistently break Rensch's rule. *PLoS ONE*, 2, e897.

Zamudio, K.R. (1998) The evolution of female-biased sexual size dimorphism: a population-level comparative study in horned lizards (*Phrynosoma*). *Evolution*, 52, 1821–1833.

Accept July 09, 2015

Table 1 SSD and body sizes in *Tribolium castaneum* populations measured. All populations assessed are wild type strains except for Rd (dominant mutation associated with altered sensory surface of the antennal club). The table includes mean body sizes in mm for both sexes (*N*=30 individuals per population and sex) and values for one index of sexual size dimorphism (see main text for details).

Strain	**Year collected**	**Country (Locality)**	**Female elytra length (mm) ± S.D.**	**Male elytra length (mm) ± S.D.**	**Lovich & Gibbons SDI**
Abidjan	unknown	Ivory Coast (Abidjan)	2.272 ± 0.049	2.235 ± 0.047	0.01566
COL-1	1987	Colombia (Palmira)	2.293 ± 0.077	2.259 ± 0.080	0.01505
CTC-485	1988	Australia (NSW)	2.143 ± 0.077	2.090 ± 0.065	0.02536
FSS2	1943	England (London)	2.331 ± 0.117	2.282 ± 0.081	0.02147
GA1	1980	USA (Georgia)	2.356 ± 0.079	2.330 ± 0.074	0.01116
Heng-5	1989	Thailand (Chiang Mai)	2.263 ± 0.071	2.207 ± 0.067	0.02537
HO-TCS	1989	Singapore (Jurong)	2.477 ± 0.071	2.396 ± 0.060	0.03381
ISR-1	1988	Israel (Tel Aviv)	2.188 ± 0.088	2.136 ± 0.083	0.02434
Japan-2	1988	Japan (Kyushu)	2.532 ± 0.074	2.383 ± 0.141	0.06253
Lab-S	1976	USA (Kansas)	2.310 ± 0.074	2.197 ± 0.054	0.05143
Montreal	1973	Canada (Montreal)	2.263 ± 0.047	2.192 ± 0.059	0.03239
PAK-1	1979	Pakistan (Peshawar)	2.248 ± 0.088	2.074 ± 0.055	0.08390
PAK-2	1979	Pakistan (Peshawar)	2.125 ± 0.060	2.087 ± 0.062	0.01821
PRC-Nanj	1989	China (Nanjing)	2.422 ± 0.072	2.371 ± 0.074	0.02151
PRC-Ning	1989	China (Ningbo)	2.414 ± 0.062	2.366 ± 0.076	0.02029
Pruz 1	1963	Poland	2.419 ± 0.059	2.327 ± 0.069	0.03954
Raj-1	<1979	India	2.316 ± 0.076	2.255 ± 0.057	0.02705
Rd	<1984	Laboratory	2.158 ± 0.074	2.053 ± 0.085	0.05114
Solet	1979	Israel (Hatzeva)	2.212 ± 0.079	2.160 ± 0.072	0.02407
Ug-3	1986	Uganda (Kampala)	2.395 ± 0.063	2.309 ± 0.070	0.03725
Z-1	1988	USA (Alabama)	2.364 ± 0.130	2.294 ± 0.128	0.03051

Fig. 1 Allometric regression plot of log female on log male body size. Each symbol denotes one laboratory stock population (strain identities provided in legend above, for further details see main text and Table 1). Regression equation: $y = 0.923 \ x + 0.040$. The hatched line of isometry (i.e. female body size = male body size) is included as a guideline. This helps illustrate a clear pattern of female-biased SSD where all points fall above the line.