Morphometry and feeding behaviour in two orthopteran species in the Kalahari (Namibia): The trait as you like it

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High entomological trait variability is supposed to reflect a combination of intra- and inter-phenotype signals. These functional signals are mirroring different behaviours and feeding habits. Our main aim was to assess if the trait distribution of body measurements of two closely related Orthoptera species is further more significantly influenced by sex (intraspecific variance) or by species (interspecific variance). To achieve this, we collected in Namibia (Africa) tettigoniids belonging to the sympatric species *Acanthoplus discoidalis* and *Acanthoplus longipes*. We measured in the field the total body length, the maximal pronotal width and length, and the third pair of legs (femur and tibia) of 106 adults. We derived the body mass and volume from empirical length and width values of the sampled specimens and compared them with literature data on African tettigoniids. The discriminant analysis shows that at species level the locomotory traits as captured by tibia and femur lengths and the size traits as captured by body and pronotal lengths clearly separate the two species. However, the intraspecific trait distributions between males and females are small in contrast to the interspecific trait distributions. We explain this latter phenomenon as consequence of dietary differences due to nitrogen contents of the host plants between *A. discoidalis* and *A. longipes*.

Keywords: *Acanthoplus* (Tettigoniidae); body size; interspecific variance; intraspecific variance; trait distribution

INTRODUCTION

The great trait variability that especially characterizes insect populations mirrors ongoing changes of morphological structures (legs, wings, abdomen, etc.) and behavioural aspects from feeding and dispersal up to reproduction and competition. Hence the importance to investigate the many functional links between behavioural modifications and morphological traits is high, both at interspecific as at intraspecific level (VIOLLE *et al*. 2012). WRIGHT's (1965) path analysis by *F*-statistics was the first attempt to elucidate the pattern and the extent of genetic variation within and among natural populations. According to ROUGHGARDEN (1972), a population's niche was suggested to be determined by the combination of two phenomena in resource use, *i.e.* an intra-phenotype variation and an inter-phenotype variation (VIOLLE *et al*. 2012).

Such results are applicable in ecology and are even more interesting if seen in the dynamic framework of interspecific competition: different sensitivity to the elemental resources use by animals with different traits allows to increase the realism of dynamic forecasting. From the perspective of a size-scaled behaviour, what PETERS asserted in 1983 remains valid: "a knowledge of allometry might allow animal behaviorists a greater definition of the probable behavior a study animal might exhibit." For instance, shifts in consumer's size in response to host plants vary and are mediated by the type and rugosity of the habitat (TEUSCHER *et al*. 2009) and also by the competition strategy employed by the insect species (AMARILLO-SUÁREZ *et al*. 2011; KALINKAT *et al.* 2015; HIRT *et al*. 2018).

Moreover, it appears that for insect movement the allometry of morphology is highly relevant (KASPARI & WEISER 2007; DIAL *et al*. 2008; WHITMAN 2008; KALINKAT *et al.* 2015; HIRT *et al*. 2017, 2018; see also TAMBURELLO *et al.* 2015 for vertebrates). Movement, behaviour and environmental conditions are therefore closely linked together. We need to examine whether, for example, resource quality favours species with larger body size, promotes species with either intrinsically faster life cycles or more opportunistic behaviours like omnivory and cannibalism (DILLON & FRAZIER 2013), and if major environmental characteristics such as different feeding strategies and habitats influence the trait distribution (MULDER & ELSER 2009).

To achieve the actual interrelationships between ecology, behaviour and functional traits, empirical data were gathered in Namibia across differently-sized sympatric species of orthopterans, *Acanthoplus discoidalis* (Walker) and *Acanthoplus longipes* (Charpentier). We checked then the validity of the statistical assumptions underlying the trait distributions, in order to verify which traits are statistically meaningful, asking the following questions:

- To what extent do shifts in the trait distribution reflect different behaviours for African tettigoniids in general and our two Namibian species in particular?
- Are trait distributions of our empirical body measurements more influenced by sex (intraspecific variance) or by species (interspecific variance)?

MATERIALS AND METHODS

During March 2012, we collected 60 adults of *Acanthoplus longipes* (30 males and 30 females) near Keetmanshoop (25°52'1.2"S, 18°06'12.5"E) and 46 adults of *Acanthoplus discoidalis* (20 males and 26 females) near Otjiwarongo (20°25'50.6"S, 16°40'10.8"E). For each specimen we measured five functional traits using a Borletti caliper (measurement error of 0.02 mm). Specifically (see Fig. 1), we measured the total Body Length (henceforth BL) from the tip of the head to the end of the abdomen (excluding the ovipositor), the maximal pronotal width (PRW) as proxy for the entire body width, the maximal pronotal length (PRL), and finally the length of femur and tibia of third pair of legs (FE_3 and TI_3, respectively) for both *A. discoidalis* (D) and *A. longipes* (L).

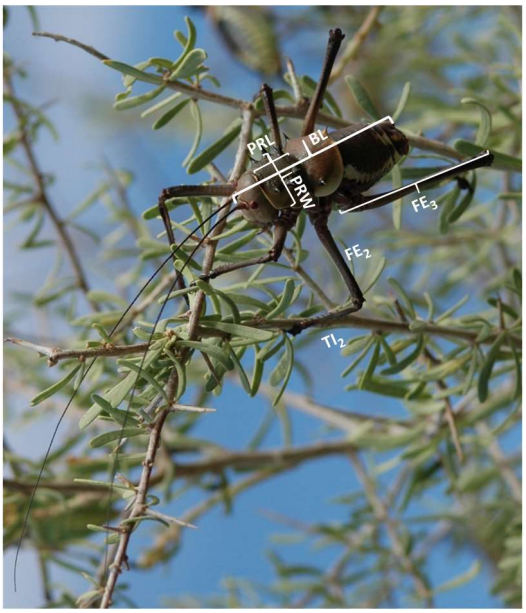

Figure 1. The field-measured functional traits for body (BL, PRW) and legs (FE_3, TI_3 not shown)

We also estimated the Body Mass (BM) of our specimens using the most comprehensive model (the so-called "Model LWTR") by SOHLSTRÖM *et al*. (2018) which considers Length, BL, Width, PRW, Taxonomic group, T, and geographic Region, R, according to the following equation:

$$\log_{10}(BM) = a_{taxon\ region} + b_{length\ region} \times \log_{10}(BL) + b_{width\ region} \times \log_{10}(PRW)$$

where

$a_{taxon} = -0.117$,

$b_{length} = 1.001$,

$b_{width} = 1.673$,

and as region the Tropics.

Also Body Volume (BV) for *Acanthoplus* was quantified using empirical data (if available) by approximating the insect shape to a box constrained by the length and the width that can control BV according to the following equation: $BV = BL \times PRW^2$ (in mm^3), where BL is and PRW the maximal pronotal width. If PRW data were unavailable, as in the case of BIDAU and MARTÍNEZ (2018) online data, we used own conversion factors to change BM into BV according to our empirical measurements:

$$BV = 81.098 \times BM - 1014.879.$$

Then, to evaluate if a functional correlation exists for the hindlegs across the African tettigoniids in general and inside the genus *Acanthoplus* in particular, we performed a regression relationship between femur length (FE_3 as shown in Fig. 1) and volume.

To test for inter- and intraspecific differences we used a one-way ANOVA using body length, length and width of pronotum and femur and tibia lengths as empirical variables and body mass estimates as derived variable ($\alpha = 0.01$). Post-hoc comparisons were conducted using the Dunnett's T3 test and we visualized the trait distribution using violin plots as realized with the "ggplot2" program by the "geom_violin()" utility in R-3.5.1. In order to evaluate the existence of any interspecific separation, we performed a Discriminant Function Analysis (DFA) considering the functional traits as empirical variables. The DFA is highly suitable to determine whether (species, sex) groups differ with regard to the mean of empirically measured trait weights (centroid), and then to use that trait to predict taxonomic group membership (*A. discoidalis* and *A. longipes*). All these statistical analyses were performed using SPSS (vers. 21 for Windows).

RESULTS

We undertake a multiple trait analysis as a function of sex and species. The comparison of males (m) and females (f) between the two species (D and L) share a remarkably significant signal for five of the six traits (Fig. 2). In particular, in the case of pronotal traits, both Dm vs. Lm and Df vs. Lf always differ at the $P < 0.001$ level. Tibia and femur lengths (TI_3 and FE_3, respectively) appear to be specie-specific as these differences are non-overlapping between *A. discoidalis* and *A. longipes* (Fig. 2, bottom). Also in this case, the ANOVAs show that both Dm vs. Lm and Df vs. Lf always differ at the $P < 0.001$ level. The correlation and regression analyses between TI_3 and FE_3 highlight strong relationships inside the genus since TI_3 as sole predictor contributes for 60% to the expected total length of the hind legs.

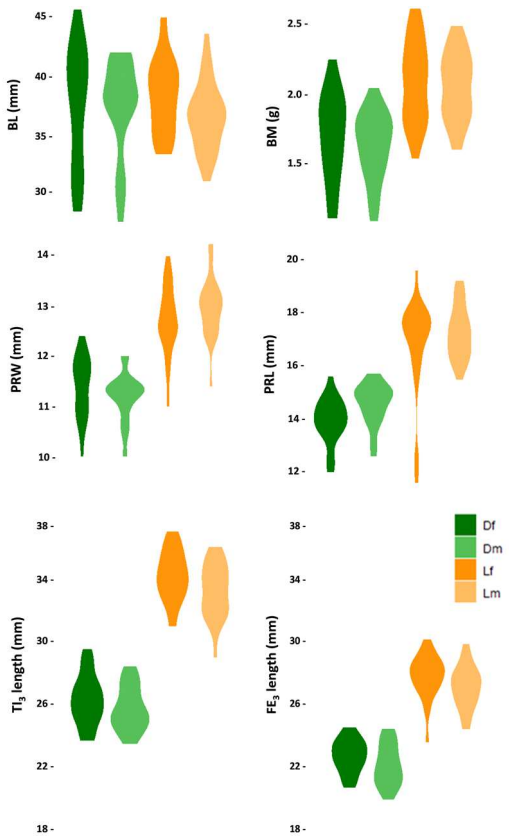

Figure 2. The trait distributions of body size and mass (upper panel), pronotal sizes (medium panel) and leg size (bottom panel) of our *Acanthoplus* specimens. Dm = *A. discoidalis*, males; Df = *A. discoidalis*, females; Lm = *A. longipes*, males; Df = *A. longipes*, females. Trait abbreviations as follows: body lenght (BL), body mass (BM), pronotal width (PRW), pronotal length (PRL), tibia length (TI_3) and femur length (FE_3). Aside BL ($F = 0.488$, $P = 0.488$ for males and $F = 0.533$, $P = 0.468$ for females), the ANOVAs of the traits remain significant between our species.

The first two discriminant functions (*DF*s) together account for 99% of the total variance (Fig. 3) and clearly separate the two species.

The first *DF* mirrors the locomotory relevance as captured by tibia and femur lengths (TI_3 = 0.897, FE_3 = 0.731) whilst the second *DF* mirrors the size traits as captured by body and pronotal lengths (BL = -0.344, PRL = 0.561). It appears clear that length traits are relevant factors in splitting our species, as the weight of the body width appears to be insignificant (Wilks' λ = 0.931, P = 0.066).

In particular, all centroids fall in distinct quadrants (Fig. 3): the males of *A. longipes* (Lm) share for both their legs and their size a *DF*>0 and fall into the first quadrant, and the females of the same species (Lf) share a *DF*>0 for the legs but a *DF*<0 for the size and fall into the second quadrant. In contrast, the females of *A. discoidalis* (Df) share for both their legs and their size a *DF*<0 and fall into the third quadrant, and the males of the same species (Dm) share a *DF*<0 for the legs but a *DF*>0 for the size and fall into the fourth quadrant.

The recently published data by BIDAU and MARTÍNEZ (2018) provided BL and FE_3 trait values for 50 African tettigoniids (25 for each sex). A multiple comparison of body mass, body length and femur length between sex resulted in highly significant ANOVAs (females BM F = 23.20, BL F = 117.17 and FE_3 F = 31.65; males BM F = 36.84, BL F = 121.73 and FE_3 F = 47.13, with P < 0.0001 in all the six cases), pointing to intraspecific variation. We also plotted the FE_3 length as function of estimated insect volume of our Namibian genus in comparison to other tettigoniids (Fig. 4).

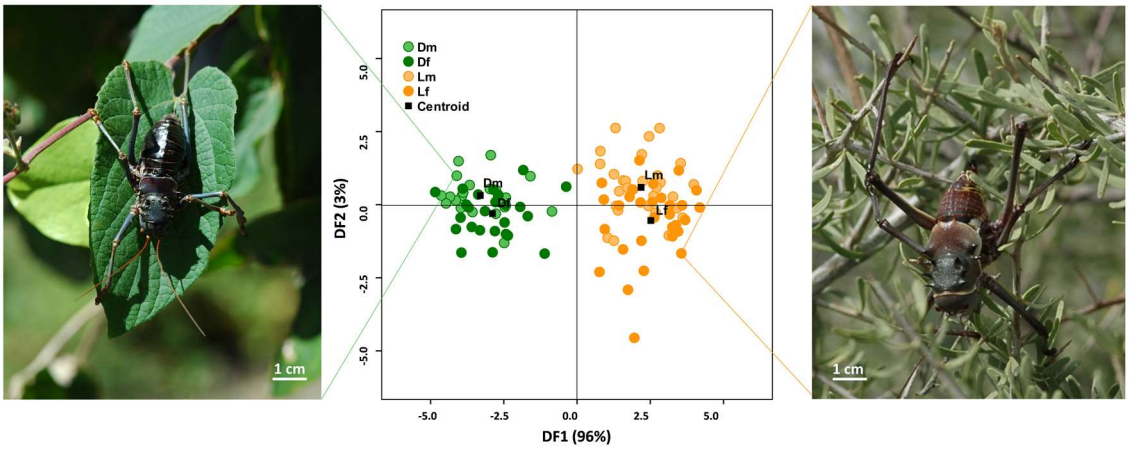

Figure 3. Scatter plot of Discriminant Function analysis based on the first two components, taking into consideration femur and tibia (*DF1*) and length traits of body and pronotum (*DF2*). Colours and symbols as in the previous figure. Specimens of *A. discoidalis* (on the left) and *A. longipes* (on the right) are shown.

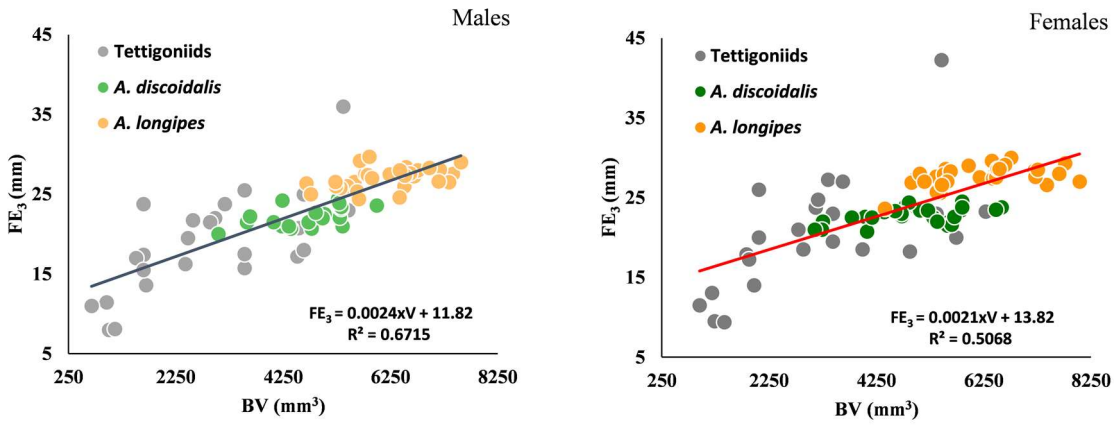

Figure 4. Allometric scaling of femur length (FE_3) as linear function of body volume (BV) in males (left panel) and females (right panel) of *Acanthoplus* specimens (same colours as in the previous figures) in comparison to African tettigoniids (grey-filled circles) as derived from the online dataset by BIDAU and MARTÍNEZ (2018).

DISCUSSION

Behaviour impasses more information than single physiological and/or morphological aspects. As it represents the answer of any individual to the changing environmental features, it is easy to suppose that a change in a behavioural pattern can result in a correspective morphological change. In most ectotherms, females are known to be longer than males (FAIRBAIRN 1997; BLANCKENHORN *et al*. 2007). Reflecting the rule by RENSCH (1959), who observed as first that any sexual size dimorphism increases with BL in the species where the males are larger than their mates and decreases with BL in the opposite case, our specimens show the same pattern. In particular, we found no statistical evidence for sexual size dimorphism of *Acanthoplus* for BL but only for BM (Fig. 2). Being this latter a function of body length and pronotal width, we are prone to think that in comparison to BL the pronotal width is the relevant trait in explaining the interspecific differences in the calculated BM values.

Foreseen breakthroughs lie in the development of novel, trait-driven frameworks for comprehensive ecological evaluation. Our study formally defines the entire trait distribution is much more separate at interspecific than at intraspecific levels and considers the implications of trait-driven behavioural patterns in these two African *Acanthoplus* species.

This may be a matter of differences in philosophy, but we consider these to have strong implications at sex level. It might in fact seem to be hard to choose a sole explanatory factor for either an enhanced locomotory efficiency or a diet-driven behaviour, but the different plants inhabited and attacked by our specimens strongly suggest the combined relevance of both explanations.

All our analyses not only unequivocally separate one species cluster from its congeneric species, but also show that our genus is much larger than other African tettigoniids. It is remarkable that tettigoniids have on average a nitrogen content of 11.2% in their tissues, much higher than the overall nitrogen content of 9.9% of other insects (FAGAN *et al*. 2002). Being even larger, our *Acanthoplus* specimens possibly demand from the foliar tissues they consume a much higher nutrient quality.

Indeed, we argue that despite their omnivory, our tettigoniids might have a remarkable difference in their food preference. The larger-sized *A. longipes* preferably feeds on *Acacia* leaves that as N_2-fixers have a much higher than average nitrogen percentage whilst the pest *A. discoidalis* is mostly feeding on cereals with a much lower nitrogen percentage (respectively 3.5% vs. 2.1% according to MULDER *et al*. 2013). This difference in nitrogen content allows our species to reach differences in mass that, in turn, needs different size in their locomotory traits.

Hence we have to discuss the extent to which traits might reflect either locomotory or feeding behaviours. As a matter of fact, the larger-sized *A. longipes* might reflect in its locomotory traits a kind of adaptation that improves the feeding possibilities by an efficient movement across the branches of the shrub they inhabit. In contrast, the shorter-sized *A. discoidalis* might reflect in its locomotory traits an attempt to compensate the high energy input due to elevate temperatures of the soil surface (cf. MARTIN *et al.* 2000) with a slower movement across cereal fields.

Summarizing, functional traits are already known to be a valuable proxy in animal and plant ecology and much more research is coming shortly. But relevant traits are much more than body size alone. Different interspecific aspects of size contribute by far the most to our discriminant analysis, highlighting the relevance of functional traits as evolutionary clumps. Shakespeare wrote "...'t is true that a good play needs no epilogue; yet to good wine they do use good bushes..." [*As You Like It*]. Notwithstanding the great potential of existing trait-based models, up to now, too many ecologists seem unaware of the benefits traits provide. Therefore, our work is not supposed to be a kind of epilogue, but a plea not to underestimate the "good bushes" by geo-tagged empirical data, existing online repositories and future trait banks (SCHNEIDER *et al.* 2019).

Our analyses convincingly demonstrate that the presumed relevance of gender-specific trait-distributions in literature is not always correct and call into question the invoked links between individual-based traits and population structures.

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REFERENCES

AMARILLO-SUÁREZ A.R., STILLWELL R.C. & FOX C.W. 2011. Natural selection on body size is mediated by multiple interacting factors: a comparison of beetle populations varying naturally and experimentally in body size. *Ecology and Evolution* 1: 1-14 (doi:10.1002/ece3.1)

BIDAU C.J. & MARTÍNEZ P.A. 2018. Evolutionary negative allometry of orthopteran hind femur length is a general phenomenon. *Zoomorphology* 137: 291-304 (doi:10.1007/s00435-018-0395-x)

BLANCKENHORN W.U., DIXON A.F., FAIRBAIRN D.J. *et al*. 2007. Proximate causes of Rensch's Rule: Does sexual size dimorphism in arthropods result from sex differences in development time? *American Naturalist* 169: 245-257 (doi:10.1086/510597)

DIAL K.P., GREENE E. & IRSCHICK D.J. 2008. Allometry of behavior. *Trends in Ecology and Evolution* 23: 394-401 (doi:10.1016/j.tree.2008.03.005)

DILLON M.E. & FRAZIER M.R. 2013. Thermodynamics constrains allometric scaling of optimal development time in insects. *PLoS One* 8: e84308 (doi:10.1371/journal.pone.0084308)

FAGAN W.F., SIEMANN E., MITTER C. *et al*. 2002. Nitrogen in insects: implications for trophic complexity and species diversification. *American Naturalist* 160: 784-802 (doi:10.1086/343879)

FAIRBAIRN D.J. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual Reviews of Ecology and Systematics* 28: 659-687 (doi:10.1146/annurev.ecolsys.28.1.659)

HIRT M.R., LAUERMANN T., BROSE U. *et al*. 2017. The little things that run: a general scaling of invertebrate exploratory speed with body mass. *Ecology* 98: 2751-2757 (doi:10.1002/ecy.2006)

HIRT M.R., GRIMM V., LI Y. *et al*. 2018. Bridging scales: Allometric random walks link movement and biodiversity research. *Trends in Ecology and Evolution* 33: 701-712 (doi:10.1016/j.tree.2018.07.003)

KALINKAT G., JOCHUM M., BROSE U. & DELL A.I. 2015. Body size and the behavioral ecology of insects: linking individuals to ecological communities. *Current Opinion in Insect Science* 9: 24-30 (doi:10.1016/j.cois.2015.04.017)

KASPARI M. & WEISER M. 2007. The size–grain hypothesis: do macroarthropods see a fractal world? *Ecological Entomology* 32: 279-282

MARTIN S.D., GRAY D.A. & CADE W.H. 2000. Fine-scale temperature effects on cricket calling song. *Canadian Journal of Zoology* 78: 706-712

MULDER C. & ELSER J.J. 2009. Soil acidity, ecological stoichiometry and allometric scaling in grassland food webs. *Global Change Biology* 15: 2730-2738 (doi:10.1111/j.1365-2486.2009.01899.x)

MULDER C., AHRESTANI F.S., BAHN M. *et al*. 2013. Connecting the green and brown worlds: Allometric and stoichiometric predictability of aboveground and belowground networks. *Advances in Ecological Research* 49: 69-175 (doi:10.1016/B978-0-12-420002-9.00002-9)

PETERS P.H. 1983. *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge, UK

RENSCH B. 1959. *Evolution above the Species Level*. Columbia University Press, New York, NY

ROUGHGARDEN J. 1972. Evolution of niche width. *American Naturalist* 106: 683-718

SCHNEIDER F.D., FICHTMUELLER D., GOSSNER M.M. *et al.* 2019. Towards an ecological trait-data standard. *Methods in Ecology and Evolution* (doi:10.1111/2041-210X.13288)

SOHLSTRÖM E.H., MARIAN L., BARNES A.D. *et al*. 2018. Applying generalized allometric regressions to predict live body mass of tropical and temperate arthropods. *Ecology and Evolution* 8: 12737-12749 (doi:10.1002/ece3.4702)

TAMBURELLO N., CÔTÉ I.M., DULVY N.K. 2015. Energy and the scaling of animal space use. *American Naturalist* 186: 196-211 (doi:10.1086/682070)

TEUSCHER M., BRÄNDLE M., TRAXEL V. & BRANDL R. 2009. Allometry between leg and body length of insects: lack of support for the size–grain hypothesis. *Ecological Entomology* 34: 718-724 (doi:10.1111/j.1365-2311.2009.01124.x)

VIOLLE C., ENQUIST B.J., MCGILL B.J. *et al.* 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology and Evolution* 27: 244-252 (doi:10.1016/j.tree.2011.11.014)

WHITMAN D.W. 2008. The significance of body size in the Orthoptera: a review. *Journal of Orthoptera Research* 17: 117-134 (doi:10.1665/1082-6467-17.2.117)

WRIGHT S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19: 395-420