Prof Tracey Chapman
School of Biological Sciences
University of East Anglia
Norwich Research Park
Norwich NR4 7TJ
United Kingdom
Email:tracey.chapman@uea.ac.uk

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Re: PhD thesis of Eoin Duffy. Investigating interactions between sexual selection and natural selection using experimental evolution in *Drosophila simulans*.

Institute of Environmental Sciences, Faculty of Biology and Earth Sciences, Jagiellonian University in Krakow

The PhD thesis by Eoin Duffy tackles the general question of the extent to which sexual selection can increase population fitness, in concert with natural selection, to aid adaptation. In addition, a second aim that becomes more evident during the thesis, and which is emphasised in Chapter 3 and in concluding remarks, is the effect of genetic variation on the strength of intersexual fitness correlations and hence the potential for intralocus sexual conflict. The aims are set against the results of several studies that have provided equivocal results. For example, some researchers report natural and selection at odds with one another but theory and empirical data relating to good genes models of sexual selection, posit the opposite. In addition researchers report negative through to positive intersexual correlations for fitness. This thesis effectively sheds light on these important discrepancies.

A brief thesis synopsis: The thesis contains a general introduction and three data chapters. The main technique is to use experimental evolution in a model fly system. **Chapter 1:** poses the question of whether sexual selection can aid adaptation (measured as population fitness) to a novel temperature. **Chapter 2**: takes individuals from the same lines and asks whether there are associated benefits on sexual traits in males. The results gave some unexpected patterns, with male sexual fitness under elevated sexual selection regimes highest in the novel environment and testis size varying according to the predictions of ecological speciation. **Chapter 3**: tests the strength of intersexual correlations for fitness assayed at 3 points following enforced inbreeding. Initial positive correlations for intersexual fitness decayed with inbreeding.

The **General introduction** starts with outlining Darwin's definition of sexual selection from the 1871 *Descent of Man*. The candidate outlines the context for the work in Chapters 1 and 2 and describes the so called good genes theory in which male quality is signalled to females by some aspect of the expression of their sexually selected traits. For example, male sexual fitness may be linked to non sexual fitness under condition dependence. In this way, if only good quality males are chosen, then the population may be purged of deleterious mutations. This is set against theory that suggests that sexual selection and natural selection oppose each other. The focus in the work in Chapter 2 is on whether, in a novel environment, fitness optima are altered to the extent that natural and sexual selection become aligned.

My questions for the candidate based on this chapter are:

- *Can the candidate discuss whether he thinks sexual and non sexual fitness can be sharply defined?*
- *Why does the candidate think that the role of sexual selection vs conflict is so divergent in* D. melanogaster vs D. simulans *and what has led to those mating systems being apparently dominated by different selection pressures? Contrasting against the apparent lack of sexual conflict is the potential existence of negative intersexual correlations revealed by the inbreeding experiments in Chapter 3.*
- *Are differences in maing systems likely to be relevant to some of the equivocal results obtained by other researchers in this general field?*

Chapter 1: sexual selection and adaptation to a novel environment in* D. simulans*.
The design here was thermal adaptation at ancestral and novel temperatures along with manipulations to sexual selection (mongamy *versus* polyandry). A reciprocal design was used to test for adaptation to each of the ennironpnments and whether this was aided or impeded by elevated sexual selection.

The design was a 4 x replicated experimental evolution at 25 degrees and the 'novel' temperature of 27 degrees. Polyandry was imposed by keeping 4 males / 1 female (60 vials per population = 240 males, 60 females), monogamy by single pairs (64 randomly chosen male-female pairs = 64 males, 64 females). The differences in the sample size are designed to equalise the effective population size. The flies were allowed 6 days of interaction then collect offspring for the next generation.

At generation 48 fitness measures (female productivity, egg-adult viability, female body size) were taken in a reciprocal design after 1 generation of standard rearing, with egg size measures taken at generation 67 to test for differential allocation to eggs.

The results showed that elevated sexual selection led to high egg to adult viability when tested in both environments. The monogamy treatment seemed to do poorly in its 'own' novel environment, can the candidate speculate on the possible reason? Sexual selection regimes also led to higher productivity except for 25 degree (ancestral) populations tested in the 27 degree (novel environments). Females from the sexual selection regimes were larger.

There wasn't always evidence of consistent adaptation, i.e. that each population has highest productivity when measured in its 'home' evolutionary environment to which it is adapted. The novel testing environments also led to higher productivity in the relaxed sexual selection treatments – this could suggest that the proximate response to temperature is stronger in these populations.

My questions for the candidate on this chapter are:

- *Can the candidate describe the strategy to equalise the effective population size, Ne?*
- *How long were the ancestral flies maintained at 25 degrees before the initiation of the novel environment of 27 degrees, were they fully adapted to 25 degrees?*
- *Can the candidate outline the rationale for using temperature as the novel selective force in relation to other novel environments that might have been used? How did the candidate account for the difference in generation time, if there was one, in the 25 and 27 degree selective regimes?*
- *What is the natural frequency of mating in this species and how do the sexual selection treatments relate to that?*

Chapter 2 Elevated sexual and natural selection improve male sexual fitness
In this chapter, the testis size and sexual competitiveness of males from the lines used in Chapter 1 was tested. The rationale was to discover whether the presence of sexual selection and natural selection (in the form of adaptation to the novel environment) reinforced each other to result in higher male sexual fitness. Broadly speaking, for male sexual fitness, the outcomes supported this idea. However, for testis size the effects were more complex.

Figure 1 attempts to outline the potential effects of a novel environment on the value of sexuall selected traits and the relative strengths of natrual and sexual selection. It isn't an especially easy figure to read and to interpret – I think it may require further elaboration, or one panel for the equilibrium situation versus one for a novel environment, this might allow the contrasts and overall directions of change to be more easily visible.

After 52 generations of selection of the regime described in Chapter 1, and following a generation of standardised rearing, male sexual fitness eas measured in a competitive settting by placing a male with 4 competitor marked males together with 1 female. These groups interacted for 7 days and the number of offspring fathered by the focal vs competitor males was then determined. At generation 67 testis size of males from the lines tested at each temperature was recorded (again after a generatin of standardised rearing). An additional set of these males were tested for attractiveness, measured as mating latency.

The results showed that males from the elevated sexual slection regimes and males evolving at the elevated temperature were both more successful at achieving paternity. A general point here is that it is better practice to describe the significant interactions prior to any main effects when describing the results. In Figure 3A,B these results are shown, though I feel that it is not really accurate to desribe the selection as ±NS, where '+' means evolving in the higher novel temperature and '–' means at the ancestral 25 degrees. It would be possible to construct a design with ± natural selection in which the opportunity for natural selection really were silenced, but this isn't what has been done here. The candidate may need to reword the presentation of the results in this respect. For the testis results, there was a significant 3 way interaction and the outcome of the influence of temperature was different when tested in each environment. It would be interesting to see how these results are affected if scaled by body size. One could compare absolute versus relative size and this might produce a clearer picture.

My questions on this chapter are:

- *To what extent does evolution in the novel environment just impose natural selection? The candidate could better justify this stark division which likely simplifies what is in effect a more complicated selective environment. Do you know the strength of natural selection imposed by the novel temperature regime, could you estimate it by loking at viability measurements? This point is discussed further on P70, top.*
- *It may also be that testis size is only a poor measure of investment in spermatogenesis – could the candidate offer an opinion on this?*
- *The results for latency are only briefly presented and would be better analysed and presented in full, as the data shown have been combined across ancestral and novel regimes. At least some of the latency measure might be attributable to male behaviour rather than female attraction?*

Chapter 3 – inbreeding alters intersexual fitness correlations in Drosophila simulans*.*
This final data chapter investigates whether inbreeding, as a mechanism to alter the structure of genetic variation within a population, can alter the strength of intralocus sexual conflict, measured

as the intersexual correlation for fitness among males and females. Inbreeding was effected by establishing isofemale lines of *D. simulans* and fitness was measured at 3 subsequent generations. The idea is to explain one factor that may explain why there is some discordance in the findings that support or not the existence of intralocus sexual conflict. The suggestion is that differences in genetic variation within populations could be associate with variation in the existence and strength of the negative intersexual correlation for fitness. For example, mutations that have sexually antagonistic effects may inflate genetic variation. In well adapted populations genetic polymorphism may be maintained by balancing selection, but in novel / or maladapted ones not so and may be dominated by alleles with effects on fitness that cut across both sexes.

Overall, the background and theory is nicely laid out in this chapter.

Genetic variation was manipulated by instigating inbreeding, with the idea that by increasing homozygote frequency would alter the intersexual correlation for fitness, with early generations showing large effects due to the expresssion of deleterious recessives, with these being purged in successive generations, leaving behind the predominately sexually antagonistic genetic variation as time proceeds.

The experiment established 40 isolines of which 33 remained for testing. Fitness estimates for each sex were made separately at generations 5, 9 and 13 of inbreeding. Female fitness was the number of offspring produced over 7 days when females from each of the lines were housed with 2 base stock males. Male fitness was scored in a competitive environment in which males from the inbred lines competed with 4 marker males for access and matings with a single marker female. The number of offspring fathered by the focal male over 7 days was again counted. A point to note is that there are potential differences in egg to adult survival for the offspring produced by focal females vs males (different densities and rearing conditions), I wonder whether there are any data that could provide insight into that.

The interesting results showed that the intersexual correlation between male and female fitness did indeed change as onbreeding progressed. These were significantly positive at the early generation and then tended to be negative, but not significantly so, after the middle and late generations of inbreeding. It is possible that the lack of statistical significance in the negative trend in medium / late inbreeding might be related to the number of lines tested – and that there might have been more power with more lines.

Questions for the candidate on this chapter are:

- *Context dependence in the measurement of fitness effects can be a problem in the accurate comparisoin of fitness effects across the sexes, can the candidate discuss this and justify the fitness leasures used?*
- *Can the candidate clarify the procedure used to put male and female fitness on the same scale. It isn't outlined in great detail on P81.*
- *Does the candidate have any estimates of the statistical power of the tests vs number of lines used, perhaps drawing from the effect sizes given in table S1.*

Concluding Remarks:
The thesis then ends with clear concluding remarks, in which the focus is on the ways in which the selective environment can be altered in a manner that affects the strength of the intersexual correlation for fitness (though this was not the main focus of how the data in Chapter 1 and 2 were presented). I'd suggest some caution in the argument offered at the top of P96, in which you

suggest that females evolving under elevated sexual selection are gaining indirect benefits of mating with attractive males. As you note, you don't have direct evidence for that.

You suggest some potential mechanisms by which male reproductive success might have been achieved on the bottom of P97 (increased sperm viability, more effective spermatogenesis). One question at this point is:

- *I wonder whether the candidate could outline how that might be tested?*

To conclude, the results contribute to our understanding of the relationship of natural and sexual selection in novel environments and of the nature of the correlation of male and female fitness in such novel environments. A number of different approaches were undertaken and the thesis presents a significant quantity of original research, which will be of broad interest to other researchers. The major contribution is in broadening our understanding of some of the complexity and context dependence of tests of the role of natural and sexual selection in adaptation, potentially resolving some of the conflicting data out there. The study on inbreeding is similarly valuable to understand the effect of the structure of genetic variation on the potential for intralocus sexual conflict.

Overall, this is a scholarly and well presented thesis, which represents a significant contribution to learning. I noted only a new typos and minor corrections needed, which are appended below. The candidate has shown themselves to be capable of conducting, analysing, interpreting and presenting original scientific research. I recommend to the Faculty of Biology and Earth Sciences of the Jagiellonian University to proceed with the steps that are necessary to confer upon Eoin Duffy the PhD degree.

Yours sincerely,

Prof Tracey Chapman

List of minor corrections:

- P6, acknowledgements – a minor point, but the last sentence in the second paragraph does not make sense.
- P16, 3 lines from the bottom, better as 'invoking' not 'evoking', same on line 2 on P94.
- P55, 8 lines from the bottom, no apostrophe needed in 'bouquet's', it is a plural – you could even just have 'bouquet'. In this sentence, the subject is also unclear, 'these' refers to bouquets, but the subject of the sentence is really male attractiveness – sentence can be rewritten for clarity.
- P55, 5 lines from bottom, should be 'selects'.
- P87, 7 lines from bottom, need a space after 'Rice &'
- P94-96 – one long paragraph, which might be better broken up into smaller units.
- P95, 5 lines from the bottom, this sentence is poorly phrased, can clarify.