COMMENTARY ON *J. GENET*. CLASSIC

Inbreeding and sex: canalization, plasticity and sexual selection

(A commentary on J. Maynard Smith, 1956. *J. Genet*. **54**, 261–279 and 497–505; reprinted in this issue as *J. Genet.* classics, pages 17–35 and 37–45)

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John Maynard Smith (1920–2004) was a very versatile evolutionary biologist. His theoretical work, with which most of us are familiar, addressed a variety of important conceptual issues, such as whether the unit of selection is typically the individual or the group, the evolutionary maintenance of sexual reproduction, the evolution of social behaviours in animals, and the likelihood of sympatric speciation being driven by host or habitat specialization, to name just a few. Relatively early in his career as a biological researcher, however, Maynard Smith also conducted extensive experimental studies on formal genetics, life-history traits, effects of inbreeding, mating behaviour and sexual selection on the fruitfly *Drosophila subobscura*, a facet of his research that is today not that well known. Several of these issues are still of interest to evolutionary geneticists, and continue to be topics of active theoretical and experimental research. In this issue, we mark the first anniversary of Maynard Smith's passing away by reprinting two of his experimental papers published in the *Journal of Genetics* in the 1950s, one dealing with inbreeding and the other with possible causes of reduced male fertility in *D. subobscura*.

An appreciation of the harmful effects of inbreeding predates our understanding of the mechanisms of heredity (e.g. Darwin 1876), and the basic population genetic consequences of breeding among related individuals were worked out early in the 20th century (Wright 1922; Haldane 1924; Fisher 1949). More recently, the consequences of inbreeding, especially with regard to the reorganization of genetic variance following population size bottlenecks (Fernandez *et al*. 1995; Lopez-Fanjul *et al*. 1999), the interaction of environment and inbreeding depression (Bijlsma *et al*. 1999; Dahlgaard and Hoffmann 2000), and the effect of inbreeding on genetic and phenotypic variance (Lopez-Fanjul and Villaverde 1989; Fowler and Whitlock 1999), have been under renewed examination. Parallel to this has been a renewed interest in the role of genotype to phenotype mapping in mediating adaptive evolutionary change (Debat and David 2001; Houle 2001), accompanied by a resurgence of interest in developmental stability and its corollaries, canalization and adaptive plasticity (Clarke 1998; Schlichting and Pigliucci 1998; Møller 1999; Gibson and Wagner 2000). Against this backdrop, it is interesting to re-read Maynard Smith's (1956b) paper on thermal acclimatization in inbred and outbred lines of *D. subobscura*, a paper that is clearly focussed on the effects of inbreeding on phenotypic variation, in terms of both canalization and adaptive plasticity, as opposed to the effects of inbreeding on trait means.

In this paper, Maynard Smith (1956b) tested a previously proposed hypothesis concerning homoeostasis, according to which outbred organisms should not only show greater developmental stability than inbred individuals in the face of environmental noise, but should also exhibit a greater degree of adaptive plasticity (Maynard Smith *et al*. 1955). Time to death at high temperature (33°C) and low humidity was assayed on few day old adults from three inbred lines of *D. suboscura*, and on three F_1 hybrids between them, after subjecting the individuals to four combinations of temperatures experienced during pre-adult development (15°C or 25°C), and early in adult life (15°C or 25°C) prior to the assay at 33°C. Prior exposure to 25°C, either during pre-adult development, or in early adult life, enhanced survival at 33°C, but the effects of acclimatization during the pre-adult and adult stages were not additive (figure 1; table 1). Maynard Smith (1956b) carried out a rather idiosyncratic analysis of these data, and hence I have reanalysed the data using a more conventional analysis of variance (ANOVA) treating replicate population as a nested factor

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within type of population, crossed with developmental temperature and adult temperature; the pattern of results is not affected by the method of analysis.

Outbred lines, as one may expect, survived longer than inbred lines at high temperature and low humidity. More interestingly, the outbred lines also showed a greater effect of acclimatization due to pre-adult exposure at 25°C, thus supporting the hypothesis of Maynard Smith *et al*. (1955). It must be noted here that the F_1 hybrids (outbred lines), unlike outbred populations kept in mass culture, are genetically homogeneous, as are the inbred lines, thus permitting phenotypic variation to be interpreted in terms of developmental stability. In this study, lines showing greater variation in time to death in a given treatment also showed a reduced effect of acclimatization, a result interpreted by Maynard Smith (1956b) as supporting the notion that lines showing greater canalization also have a greater capacity for adaptive plasticity, and vice versa. It is, however, possible to argue that the correlation between capacity for canalization and plasticity does not reflect a causal relationship. Nevertheless, the possibility of a relationship between the ability to maintain developmental homoeostasis and the ability to alter phenotypes in an adaptive manner is an intriguing one that certainly merits further study. Recent evidence for the role of heat shock proteins in maintaining phenotypic stability (Rutherford and Linquist 1998), together with studies showing the up regulation of heat shock proteins even under non-stressful conditions in inbred lines (Kristensen *et al*. 2002; Pedersen *et al*. 2005), suggest that heat shock proteins also react to intrinsic (genetic) stress due to inbreeding, perhaps as a response to some abnormality in protein structure. Interestingly, the up regulation of heat shock proteins in inbred lines does not appear to enhance their thermal tolerance, suggesting that there are many subtleties to this story of inbreeding and thermal tolerance, first examined by Maynard Smith (1956b), that are yet to be unravelled.

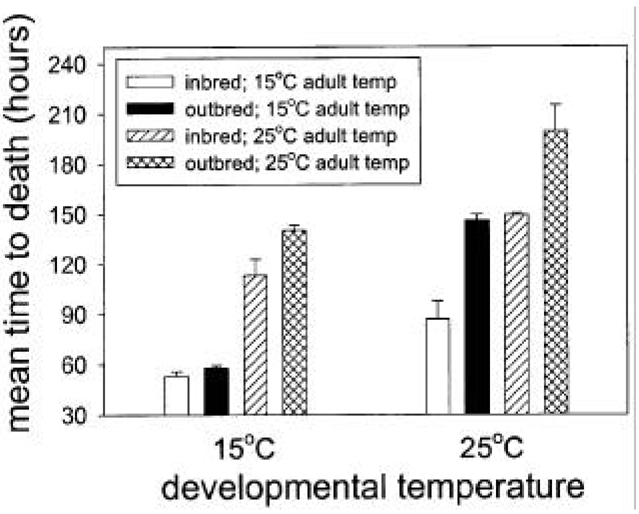

Figure 1. Mean (± s.e.) time to death at 33°C in inbred versus outbred populations of *Drosophila subobscura* subjected to all cross combinations of developmental and adult temperatures of 15°C and 25°C, respectively. Error bars are based on the variation among three replicate populations of each type (Figure based on data in Table 1 of Maynard Smith 1956b).

The second paper of Maynard Smith (1956a) reprinted in this issue is an interesting example of a research manuscript that mirrors the way a study was conceived of and turned out, as opposed to "standard" papers which report neat stories that are often presented in a way very different from how they developed. The study was conducted to examine the causes for very poor and variable male fertility in a particular line of *D. subobscura*, especially whether male fertility was significantly affected by the age of the male at mating, time elapsed since the male's previous mating, or the period elapsed between mating and time of egg collection. The major result in response to this question was that males of that line produced less and poorer quality sperm than those of other, normally fertile, lines. What is more interesting are the somewhat tangential observations on courtship behaviour – and how it is affected by inbreeding – as well as the inferences drawn about the operation of sexual selection such that females choose males that will enhance their (the females') fitness. These are topics still controversial and important in evolutionary genetics. For example, there is now increasing support for the notion that many apparent instances of female choice of mates are actually due to certain males being better at outcompeting other males for matings, and that mating

Table 1. Summary of ANOVA on time to death at 33°C in inbred versus outbred populations of *Drosophila subobscura* subjected to all cross combinations of developmental and adult tempratures of 15°C and 25°C, respectively (Reanalysis of data in table 1 of Maynard Smith 1956b).

Effect	*df*	MS	*F*	*p*
Type (inbred vs. outbred)	1	206.9	35.94	< 0.01
Developmental temperature	1	206.2	85.49	< 0.01
Adult temperature	1	186.4	134.41	< 0.01
Type × Dev. temp.	1	206.2	10.92	0.029
Type × Adult temp.	1	186.4	0.35	0.587
Dev. temp. × Adult temp.	1	146.7	1.84	0.246
Type × Dev. temp. × Adult temp.	1	146.7	2.54	0.186

with such males may actually be detrimental rather than beneficial to the female's fitness (Holland and Rice 1998, 1999; Gavrilets *et al*. 2001; Friberg and Arnquist 2003). In hindsight, it is rather likely that Maynard Smith's (1956a) argument that females prefer outbred males over inbred males because outbred males are more fertile, is wrong. Indeed, as he noted in a wonderfully detailed description of courtship in these populations, in a passage that would probably have been edited out in a more contemporary manuscript, "It seems therefore that the lower mating success of inbred males is due, not so much to a lower intensity of courtship, as to lower athletic ability. This term is intended to include those capacities which in human beings contribute to athletic success, namely, physiologically efficient muscles, sense organs and neuromuscular co-ordination. The spirit is willing but the flesh is weak".

Another interesting side issue raised in this paper is that of the contributions of testicular versus accessory gland secretions to the behaviour of mated females, especially their lack of readiness to mate again. Maynard Smith (1956a) suggests that it is insemination rather than copulation that renders females refractory, although the data are not very clear. Understanding the role of accessory gland secretions in mediating costs of mating, sperm competition and female reproductive behaviour is presently an active area of research, as can be seen from the review of Singh *et al*. (2002). Once again, one finds Maynard Smith addressing an issue – albeit in passing – that is still a question of considerable importance in genetics and evolution. It was, indeed, this ability to focus on important issues and to dissect and discuss them with exemplary clarity that characterized Maynard Smith's long and distinguished career in evolutionary genetics.

References

Bijlsma R., Bundgaard J. and Van Putten W. F. 1999 Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* **12**, 1125–1137.

Clarke G. M. 1998 Developmental stability and fitness: The evidence is not quite so clear. *Am. Nat.* **152**, 762–766.

Dahlgaard J. and Hoffmann A. A. 2000 Stress resistance and environmental dependency of inbreeding depression in *Drosophila melanogaster*. *Conserv. Biol.* **14**, 1187–1192.

Darwin C. 1876 *The effects of crossing and self-fertilization in the vegetable kingdom*. John Murray, London.

Debat V. and David P. 2001 Mapping phenotypes: canalization, plasticity and developmental stability. *Trends Ecol. Evol.* **16**, 555–561.

Fernandez A., Toro M. A. and Lopez-Fanjul C. 1995 The effect of inbreeding on the redistribution of genetic variance of fecundity and viability in *Tribolium castaneum*. *Heredity* **75**, 376–381.

Fisher R. A. 1949 *The theory of inbreeding*, 1st ed. Oliver and Boyd, Edinburgh.

Fowler K. and Whitlock M. C. 1999 The distribution of phenotypic variance with inbreeding. *Evolution* **53**, 1143–1156.

Friberg U. and Arnquist G. 2003 Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. *J. Evol. Biol.* **16**, 797–811.

Gavrilets S., Arnquist G. and Friberg U. 2001 The evolution of female mate choice by sexual conflict. *Proc. R. Soc. Lond. B* **268**, 531–539.

Gibson G. and Wagner G. 2000 Canalization in evolutionary genetics: a stabilizing theory? *BioEssays* **22**, 372–380.

Haldane J. B. S. 1924 The mathematical theory of natural and artificial selection. Part II. The influence of partial self-fertilization, inbreeding, assortative mating, and selective fertilization on the composition of Mendelian populations, and so on natural selection. *Proc. Cambr. Philos. Soc. Biol. Sci.* **1**, 158–163.

Holland B. and Rice W. R. 1998 Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* **52**, 1–7.

Holland B. and Rice W. R. 1999 Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl. Acad. Sci. USA* **96**, 5083–5088.

Houle D. 2001 The character problem in life history evolution. In *The Character Concept in Evolutionary Biology* (ed. G. P. Wagner), pp. 109–140. Academic Press, San Diego.

Kristensen T. N., Dahlgaard J. and Loeschcke V. 2002 Inbreeding affects Hsp70 expression in two species of *Drosophila* even at benign temperatures. *Evol. Ecol. Res.* **4**, 1209–1216.

Lopez-Fanjul C. and Villaverde A. 1989 Inbreeding increases genetic variance for viability in *Drosophila melanogaster*. *Evolution* **43**, 1800–1804.

Lopez-Fanjul C., Fernandez A. and Toro M. A. 1999 The role of epistasis in the increase in the additive genetic variance after population bottlenecks. *Genet. Res.* **73**, 45–59.

Maynard Smith J. 1956a Fertility, mating behaviour and sexual selection in *Drosophila subobscura*. *J. Genet.* **54**, 261–279.

Maynard Smith J. 1956b Acclimatization to high temperatures in inbred and outbred *Drosophila subobscura*. *J. Genet.* **54**, 497–505.

Maynard Smith J., Clarke J. M. and Hollingsworth M. J. 1955 The expression of hybrid vigour in *Drosophila subobscura*. *Proc. R. Soc. Lond. B* **144**, 159–171.

Møller A. P. 1999 Developmental stability is related to fitness. *Am. Nat.* **153**, 556–560.

Pedersen K. S., Kristensen T. N. and Loeschcke V. 2005 Effects of inbreeding and rate of inbreeding in *Drosophila melanogaster* – Hsp70 expression and fitness. *J. Evol. Biol.* (in press), doi: 10.1111/j.1420–9101.2005.00884.x.

Rutherford S. L. and Lindquist S. 1998 Hsp90 as a capacitor for morphological variation. *Nature* **396**, 336–342.

Schlichting C. D. and Pigliucci M. 1998 *Phenotypic evolution: a reaction norm perspective*. Sinauer, Sunderland.

Singh S. R., Singh B. N. and Hoenigsberg H. F. 2002 Female remating, sperm competition and sexual selection in *Drosophila*. *Genet. Mol. Res.* **1**, 178–215.

Wright S. 1922 The effects of inbreeding and crossbreeding on guinea pigs. III. Crosses between highly inbred families. *USDA Bull.* **1121**, 1–60.