Genital damage, kicking and early death

The battle of the sexes takes a sinister turn in the bean weevil.

Because the costs and benefits of polygamy differ for males and females, copulation is not always a cooperative venture between the sexes[1]. Sperm competition[2] can build on this asymmetry, producing male traits that harm females[3,4], thereby generating coevolutionary arms races between the sexes[5]. We have found that the male genitalia of the bean weevil *Callosobruchus maculatus* damage the female genitalia, and that females act to reduce the extent of this damage. We propose that these functionally diametric sexual traits form the basis of reproductive conflict.

The intromittent organ of male *C. maculatus* bears strongly sclerotized spines (Fig. 1) which are everted in the central region of the female's genital tract during copulation. Male *C. maculatus* do not remove the sperm of other males as a mechanism of sperm competition[6], so it is unlikely that the spines

are used for this, as they are in other insect species[7].

Examining the *in situ* genitalia of copulating pairs, we found that these sclerotized spines penetrated the cuticular lining of the female's genital tract (Fig. 2a). Once-mated females had repaired punctures (melanized haemocyte plugs) in the endocuticular lining of their genital tracts 16 hours after copulation (Fig. 2b) which were absent in virgins (Fig. 2c). (In virgins, the total area of melanized 'wound' tissue was 0 ± 0 μm^2, $n = 20$; in once-mated females, the mean extent of repair was 112.75 ± 69.6 μm^2, $n = 20$; $U = 400$, $P < 0.0001$.) The spines on the intromittent organ of male *C. maculatus* therefore damage the female genital tract

during copulation, a phenomenon that may occur in other insects[8].

Female *C. maculatus* repeatedly kick males towards the end of copulation[6]. We prevented females from kicking their mates by ablating the kicking legs, and then compared their mean copulation duration with that of a treatment control group (ablated non-kicking legs) and an unmanipulated control group. Non-kicking females copulated for significantly longer (866.9 ± 1.07 s) than treatment control (501.3 ± 1.06 s) and unmanipulated control (588.8 ± 1.08 s) groups (log-transformed data, analysis of variance, $F = 14.16$, d.f. $= 2{,}69$, $P < 0.001$; Tukey, $P < 0.001$ for each comparison between non-kicking and control groups;

Figure 1 Scanning electron micrograph of the male bean weevil's intromittent organ showing the arrangement of sclerotized spines on the tip. The spines unfold in the region of the female genital tract shown in Fig. 2. The intromittent organ is about 800 µm long. Micrograph by Andrew Syred, Microscopix.
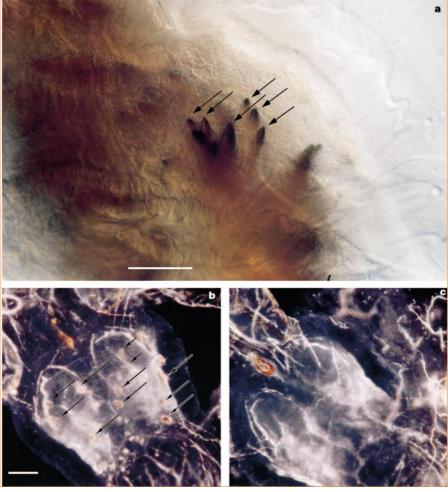

Figure 2 The anatomy of reproduction in *Callosobruchus maculatus*. **a,** Normarsky micrograph of the epithelial surface of the female genital tract containing the male intromittent organ: the specimen was dissected from a copulating pair snap-frozen in liquid nitrogen. After freezing, the *in copula* genitalia were removed and examined with a compound microscope (Leitz Diaplan). The photomicrographic image is focused on the epithelial surface of the female's genital tract: the arrows indicate the tips of spines that are in, and have passed through, this focal plane and so have penetrated the cuticular lining of the female's genital tract. **b,** A dark-field micrograph of the distal portion of a female genital tract 16 h after a single copulation. The arrows indicate melanized haemocyte plugs in the endocuticle. Anterior is towards the top left-hand corner in all micrographs. Scale bars, 30 µm. **c,** A dark-field micrograph of a dissection of the distal portion of a virgin female's genital tract.

control group means did not differ).

Mate-kicking by females therefore shortens copulation. Moreover, females that kicked during copulation sustained significantly less damage to their genital tracts (147.06 ± 84.9 μm^2) compared with females that were unable to kick (272.4 ± 142 μm^2) (log-transformed data, $t = -2.52$, d.f. = 38, $P < 0.02$).

If genital-tract damage is costly for females, there is probably a reproductive conflict over the frequency of copulation in *C. maculatus*. To determine whether copulation frequency has a life-history cost, we measured the longevity of singly and doubly mated females that were maintained in isolation with a limited number of oviposition sites to control egg-production costs (there was no difference in fecundity between treatments; $t = 0.009$, d.f. = 89, $P > 0.05$). Doubly mated females died significantly younger than singly mated females (log-rank Mantel-Cox, $\chi^2 = 5.42$, d.f. = 1, $P < 0.02$), indicating that remating reduces longevity, although reduced longevity does not necessarily result in reduced female fitness. The higher death rate of remated females could be due to genital damage incurred during copulation.

There are at least two, non-exclusive ways in which genital wounding could increase the fitness of male *C. maculatus*. First, it may reduce the risk of sperm competition[9] if females postpone remating to delay receiving more damage to their genitalia. This may increase the number of eggs fertilized by the last male to mate. Second, genital damage may increase immediate oviposition rates because females perceive genital damage as a threat to survival and invest more in current reproduction; also, males transfer compounds that increase the rate of female egg-laying (as occurs in the fruitfly *Drosophila melanogaster*[10]) and which might enter the female's haemolymph rapidly through the genital punctures. An extended interval between female matings and/or increased oviposition rates will benefit the last male to copulate, as he will achieve sperm precedence[6].

An antagonistic male trait — the transfer of ejaculatory compounds that alter female behaviour and reduce female longevity — has been documented in *D. melanogaster*[3,10], but it is unclear how female *D. melanogaster* respond to the selection pressure arising from the resultant reproductive conflict. Female *C. maculatus* seem to have responded to male-induced genital wounding by kicking their partner during copulation. This behaviour reduces the damage inflicted on their genital tract, and may therefore represent a counteradaptation to a potentially antagonistic male reproductive trait.

Helen S. Crudgington, Mike T. Siva-Jothy
Department of Animal & Plant Sciences,
University of Sheffield, Sheffield S10 2TN, UK
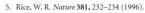*e-mail: m.siva-jothy@sheffield.ac.uk*

1. Bateman, A. J. *Heredity* **2**, 349–368 (1948).
2. Parker, G. A. *Biol. Rev.* **45**, 525–567 (1970).
3. Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. *Nature* **373**, 241–244 (1995).
4. Arnqvist, G. & Rowe, L. *Proc. R. Soc. Lond. B* **261**, 123–127 (1995).
5. Rice, W. R. *Nature* **381**, 232–234 (1996).
6. Eady, P. *Ecol. Entomol.* **19**, 11–16 (1994).
7. Waage, J. K. *Science* **203**, 916–918 (1979).
8. Merritt, D. J. *Zoomorphology* **108**, 359–366 (1989).
9. Simmons, L. W. & Siva-Jothy, M. T. in *Sperm Competition and Sexual Selection* (eds Birkhead, T. R. & Møller, A. P.) 341–432 (Academic, London, 1998).
10. Baumann, H. *J. Insect Physiol.* **20**, 2347–2363 (1974).

Environmental chemistry

Is acidification still an ecological threat?

There has been a significant reduction in anthropogenic acid deposition in Europe and North America, and now we need to gauge the rate and extent of ecosystem recovery. Stoddard *et al.*[1] have reported a widespread aquatic recovery from acidification in European ecosystems in response to a fall in sulphate deposition. But many sites in central Europe are showing a significant delay in aquatic recovery from acidification, or even no recovery at all, and only some of them show biological recovery of waters or a recovery from soil acidification. Ecosystem management still needs to consider the consequences of acidification.

Results obtained in Europe from monitoring network programmes such as ICP-waters[1] (for International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes) may bias overall conclusions because these programmes tend to focus on areas with high sensitivity to acid input. This high sensitivity is caused primarily by a low storage capacity for sulphate and protons (that is, soils that are characterized by all or a combination of: shallow post-glacial soil development, sandy soil texture, a high humus content). Data from soils with a high storage capacity for sulphate and protons indicate that reversing water acidification can take decades, because the release of previously stored sulphate causes cation leaching and acidification of deeper soil layers and waters.

Modelling by linear-regression analysis shows that the pH and alkalinity[2] of the streams at the German catchments Lehstenbach, in Fichtelgebirge, northeastern Bavaria, and Lange Bramke, in the Harz mountains, Lower Saxony, are not increasing — even though sulphate and
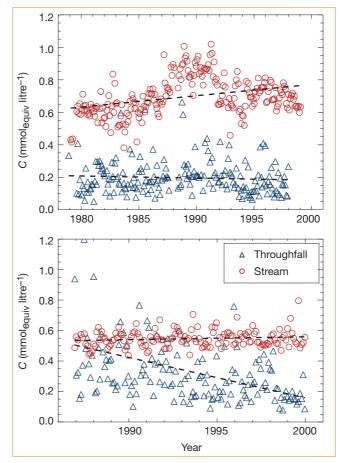

Figure 1 Nutrient cation concentration (where C represents the sum of the Ca^{2+}, Mg^{2+}, K^+ and Na^+ ion concentrations) in throughfall (precipitation below forest canopy; triangles) and streams (circles) at the catchments Lange Bramke (top) and Lehstenbach (bottom) in Germany. Black dashed lines represent a linear regression and indicate the overall trend in C over the study period.