measured long after the excavations, however, appropriate on-site dosimetry was not available and it was suggested that these ESR dates underestimate the age of the teeth[24]. Although a date of about 200 kyr is acceptable for the end of the Acheulo-Yabrudian, the dates from Tabun and Qesem caves indicate a very long and dynamic cultural complex covering about 200 kyr between the two major Palaeolithic complexes, the Lower Palaeolithic Acheulian and the Middle Palaeolithic Mousterian.

Our study is the first to date Acheulo-Yabrudian deposits by the method of $^{230}Th/^{234}U$ TIMS. On the basis of samples extracted during the archaeological excavation, an exact stratigraphic association of the dates can be made. Three main points emerge from the findings. First, the Acheulo-Yabrudian complex probably started well before 382 kyr ago, presumably during oxygen isotope stage 11. Because we have not yet dated the lower parts of the sediments in Qesem Cave, it would be fair to assume dates that accord well with the early Tabun E dates up to 400 kyr. Second, Acheulo-Yabrudian sediments at Qesem Cave are covered by a speleothem dated to 152 kyr. The Acheulo-Yabrudian occupation in Qesem Cave therefore ceased long before that date. Third, the dates of Qesem Cave represent the last cultural phase of the Lower Palaeolithic, the Acheulo-Yabrudian complex, supporting a maximum age limit of about 207 kyr ago for the earliest stages of the Middle Palaeolithic Mousterian complex. No traces of Mousterian occupation were found at Qesem.

The rich Acheulo-Yabrudian deposits at Qesem Cave offer a rare opportunity to study human adaptation and evolution in the Middle Pleistocene. Because the dates indicate that human activity occurred mostly before 382 kyr, and because the site is located within the 'out-of-Africa' corridor, the information obtained by a study of Qesem Cave is likely to contribute substantially to our understanding of the origins and dispersal of modern humans[2]. The Levantine Acheulian assemblages predating the Acheulo-Yabrudian were probably made by *Homo erectus* (*sensu lato*), whereas Mousterian industries postdating the Acheulo-Yabrudian were made by both anatomically modern humans and *Homo neanderthalensis.* It would be interesting to learn who was the maker of the unique Acheulo-Yabrudian assemblages[3]. If human remains are recovered, Qesem might hold a key to the understanding of evolution and dispersal of modern humans. The stratigraphically distinct archaeological horizons at Qesem have already provided, and will continue to provide, information on the late Lower Palaeolithic Acheulo-Yabrudian lithic variability. Such knowledge will improve our understanding of technological innovations, such as the beginning of the systematic production of blades in the Levant[4,6] and the early stages of the Levallois technique, a technological breakthrough that became globally prominent in the Middle Palaeolithic. In contrast, this period also saw the final stages of hand-axe manufacture, a tradition that had accompanied humans for about one and a half million years. □

Received 25 March; accepted 23 April 2003; doi:10.1038/nature01718.

1. Bar-Yosef, O. The Lower Paleolithic in the Near East. *J. World Prehist.* **8,** 211–265 (1994).
2. Bar-Yosef, O. in *Origins of Anatomically Modern Humans* (eds Nitecki, M. H. & Nitecki, D. V.) 23–66 (Plenum, New York, 1994).
3. Stringer, C. Modern human origins: progress and prospects. *Phil. Trans. R. Soc. Lond. B* **357,** 536–579 (2002).
4. Ronen, A. (ed.). *The Transition from Lower to Middle Paleolithic and the Origins of Modern Man* (BAR International Series 151, Oxford, 1982).
5. Copeland, L. in *Towards Modern Humans: Yabrudian and Micoquian, 400–50 kyears ago* (eds Ronen, A. & Weinstein-Evron, M.) 97–117 (BAR International Series 850, Oxford, 2000).
6. Vishnyatsky, L. B. 'Running ahead of time' in the development of Paleolithic industries. *Antiquity* **68,** 134–140 (1994).
7. Frumkin, A. in *The Nahal Qanah Cave* (ed. Gopher, A.) 139–146 (Tel Aviv Univ., Tel Aviv, 1996).
8. Frumkin, A., Ford, D. C. & Schwarcz, H. P. Continental oxygen isotopic record of the last 170,000 years in Jerusalem. *Quat. Res.* **51,** 317–327 (1999).
9. Kaufman, A. *et al.* U-Th isotope systematics from the Soreq Cave, Israel and climatic correlations. *Earth Planet. Sci. Lett.* **156,** 141–155 (1998).
10. Garrod, D. A. E. & Bate, D. M. A. *The Stone Age of Mount Carmel* Vol. I (Clarendon, Oxford, 1937).
11. Garrod, D. A. E. Acheuléo-Jabrudian et 'Pré-Aurignacien' de la grotte du Taboun (Mont Carmel): Étude stratigraphique et chronologique. *Quaternaria* **3,** 39–59 (1956).
12. Jelinek, A. J. in *The Emergence of Modern Humans* (ed. Mellars, P.) 81–90 (Edinburgh Univ. Press, Edinburgh, 1990).
13. Rust, A. *Die Höhlenfunde von Yabrud (Syrien).* (Neumunster, 1950).
14. Lauritzen, S.-E. & Lundberg, J. Calibration of the speleothem delta function: An absolute temperature record for the Holocene in northern Norway. *The Holocene* **9,** 659–670 (1999).
15. Lauritzen, S.-E. & Lundberg, J. *TIMS Age 4U2U: A Program for Raw Data Processing, Error Propagation and $^{230}Th/^{234}U$ Age Calculation for Mass Spectometry. Turbo Pascal Code* (Department of Geology, Bergen Univ., Bergen, 1997).
16. Schwarcz, H. P. Absolute age determination of archaeological sites by uranium series dating of travertines. *Archaeometry* **22,** 3–24 (1980).
17. Turville Petre, F. *Research in Prehistoric Galilee 1925–1926 and a Report on the Galilee Skull* (British School of Archaeology in Jerusalem, London, 1927).
18. Gissis, I. & Bar-Yosef, O. New excavations in Zuttiyeh cave, Wadi Amud, Israel. *Paléorient* **5,** 175–180 (1974).
19. Sohn, S. & Wolpoff, M. H. Zuttiyeh face: A view from the East. *Am. J. Phys. Anthropol.* **91,** 325–337 (1993).
20. Vandermeersch, B. in *The Human Revolution* (eds Mellars, P. & Stringer, C.) 155–164 (Edinburgh Univ. Press, Edinburgh, 1989).
21. Zeitoun, V. The taxonomical position of the skull of Zuttiyeh. *C.R. Acad. Sci. Paris* **332,** 521–525 (2001).
22. Weinstein-Evron, M., Tsatskin, A., Porat, N. & Kronfeld, J. A Th/U date for the Acheulo-Yabrudian layer in the Jamal cave, Mount Carmel, Israel. *S. Afr. J. Sci.* **95,** 186–188 (1999).
23. Bar-Yosef, O. in *Neandertals and Modern Humans in Western Asia* (eds Akazawa, T., Aoki, K. & Bar-Yosef, O.) 39–56 (Plenum, New York, 1998).
24. Mercier, N., Valladas, H. G., Froget, L., Joron, J.-L. & Ronen, A. Datation par thermoluminescence de la base du gisment Paléolithique de Tabun (mont Carmel, Israel). *C.R. Acad. Sci. Paris* **330,** 731–738 (2000).
25. Ronen, A., Shifroni, A., Laukhin, S. & Tsatskin, A. *À la Recherche de l'Homme Préhistorique, Etudes et Recherches Archaéologiques de L'Université de Liège*, **95,** 209–224 (Université de Liége, Liége, 2000).
26. Schwarcz, H. P., Goldberg, P. D. & Blackwell, B. Uranium series dating of archaeological sites in Israel. *Israel J. Earth Sci.* **29,** 157–165 (1980).
27. Porat, N., Chazan, M., Schwarcz, H. & Kolska Horwitz, L. Timing the Lower to Middle Paleolithic boundary: New dates from the Levant. *J. Hum. Evol.* **43,** 107–122 (2002).
28. Grün, R., Stringer, C. & Schwarcz, H. ESR dating of teeth from Garrod's Tabun cave collection. *J. Hum. Evol.* **20,** 231–248 (1991).
29. Rink, W. J. *et al.* Age of the Middle Paleolithic site of Rosh Ein Mor, Central Negev, Israel: Implications for the age range of the Early Levantine Mousterian of the Levantine corridor. *J. Archaeol. Sci.* **30,** 195–204 (2002).

Acknowledgements We thank N. Goren-Inbar, S. Rosen and S. Weiner for their comments on the original manuscript, and H. C. Linge and F. Saez for U–Th TIMS analysis. The Qesem Cave research project is supported by the Israel Science Foundation, the L.S.B. Leakey Foundation and the CARE Archaeological Foundation.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for material should be addressed to R.B. (barkaran@post.tau.ac.il).

The evolution of reproductive isolation through sexual conflict

Oliver Y. Martin & David J. Hosken

Zoological Museum, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Classical population-genetics theory suggests that reproductive isolation will evolve fastest in small isolated populations[1]. In contrast, recent theory suggests that divergence should occur fastest in larger allopatric populations[2]. The rationale behind this is that sexual conflict, potentially the strongest driver of speciation, is greater in larger, higher-density populations. This idea is highly controversial[3] and has little experimental support[4,5]. Here we show, using replicate fly populations with varying levels of sexual conflict, that larger, more dense populations with more sexual conflict diverged to a greater degree than small populations with relaxed conflict. This result strongly suggests that speciation can occur rapidly in large populations through increased sexual conflict.

Sexual conflict is a potent evolutionary force that may lead to

cycles of sexually antagonistic coevolution, with adaptations in one sex provoking counter-adaptations in the other[6,7]. These antagonistic cycles are implicated in the rapid evolution of some reproductive proteins, the fastest-evolving proteins known[7,8]. Sexually antagonistic coevolution is particularly interesting for its potential to drive fast evolutionary change and cause allopatric population divergence[9]. This occurs because reproductive genes evolve under sexual conflict, and hence during periods of allopatric divergence, reproductive incompatibilities should swiftly accumulate. This mechanism is considered to be a widely applicable sexual-selection speciation engine[10].

To investigate population divergence resulting from sexual conflict, we used experimental evolution with replicate populations of the dung fly *Sepsis cynipsea*. We used three treatments: high density, low density and monogamy. In high-density and low-density populations, flies were kept in large containers and could interact freely so that both pre- and postcopulatory sexual selection, including sexual conflict, were possible. Increasing numbers of compatible males in larger populations caused increased conflict in theoretical investigations[2]. Here it is the number of sexual interactions *per se*, but nonetheless, in the model[2] and here, conflict was elevated at higher population density. Monogamous populations consisted of pairs with lifelong monogamy and random pairing within lines, and represent populations where sexual conflict was absent. After 35 generations of evolution under different levels of conflict followed by two generations of relaxed selection, population divergence was assessed based on mating behaviour. Flies were paired either within or between populations but always within treatments. Behaviour was analysed with the proportion of copulations or reluctance behaviour as dependent variables, and selection (monogamous, low density or high density) and mating type (within or between (populations of the same treatment)) as predictors. As shown in Fig. 1, there were highly significant effects of both predictors and their interaction on the proportion of copulations (selection: $F_{2,12} = 147.28$, $P = 0.0001$; mating type: $F_{1,12} = 63.54$, $P = 0.0001$; selection × mating type: $F_{2,12} = 27.55$, $P = 0.0001$). Higher proportions of successful copulations were found in focal pairs from monogamous populations compared with those from the conflict treatments (low-density and high-density populations). Additionally, there was no difference between the mating proportions in pairs formed from within or between allopatric monogamous populations (Fig. 1). In contrast, there were pronounced differences in the conflict treatments. Copulation frequency was greater in pairings within conflict populations than those between populations, and this effect was stronger in high-density than in low-density populations (Fig. 1). Analysis of female resistance behaviour (proportion of females shaking to dislodge males) confirms this (Fig. 2). More successful cross types (those with more copulations) are also those where female resistance occurs more rarely (selection: $F_{2,12} = 137.38$, $P = 0.0001$; mating type: $F_{1,12} = 127.97$, $P = 0.0001$; selection × mating type: $F_{2,12} = 37.60$, $P = 0.0001$; Fig. 2). Notably, this demonstrates that low copulation levels are not due to lack of male attempts, but rather to rejection by females. These results indicate that copulation frequency decreased and female resistance behaviour increased with increasing sexual conflict. Furthermore, in pairings between different populations (within conflict treatments), females showed greater resistance and copulated less than within populations, indicating female preference for males from their own population. Why females are more reluctant to mate with males from other conflict populations is uncertain. Presumably there is fitness variation underlying this behaviour, although this remains to be explored.

Consistent with theory[2,11] there was no indication of increased reproductive isolation between allopatric monogamous populations. Divergence was not expected to be as pronounced in the absence of conflict (that is, under monogamy). Finally, the effects on copulation and female resistance frequency were more pronounced in crosses between different high-density populations than in those between low-density populations (Post-hoc testing of the main models using Fisher's partial least significant difference (PLSD); high density (between) versus low density (between): copulation, $P = 0.0001$; shaking, $P = 0.0006$; Figs 1 and 2). In both low-density and high-density populations, frequencies of copulation were significantly lower and female resistance more pronounced in crosses between populations than within populations. However, when females were crossed within their own populations, high-density and low-density populations did not differ significantly from each other (Fisher's PLSD; high density (within) versus low density (within): copulation, $P = 0.39$; shaking, $P = 0.07$). These results indicate that increased behavioural (precopulatory) reproductive isolation has evolved between high-density compared with between low-density or monogamous populations, and indicates that population density had a role in driving reproductive isolation by intensifying sexual conflict[2]. Unlike in the model[2], however, divergence in our populations must have occurred using standing genetic variation rather than new mutations.

Comparative evidence also indicates that conflict leads to increased speciosity[12], although this has been contested[13]. Nevertheless, the experimental results presented here demonstrate the

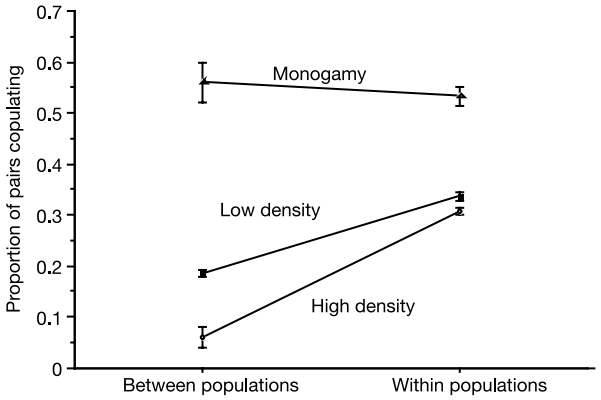

Figure 1 Interaction plot showing the effects of treatment (monogamy, low-density or high-density populations) and mating type (within populations or between populations of the same treatment) on the proportion of pairs that copulated. Error bars indicate ± 1 standard error.

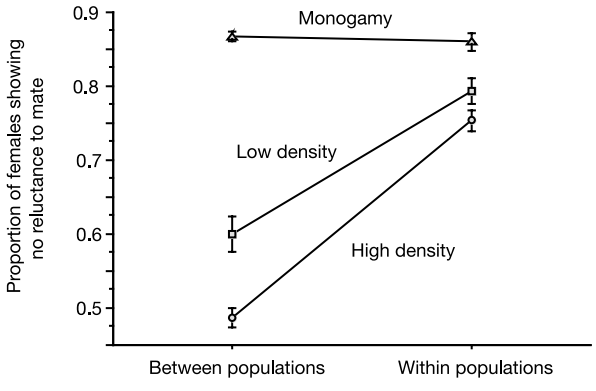

Figure 2 Interaction plot showing the effects of treatment (monogamy, low-density or high-density populations) and mating type (within populations or between populations of the same treatment) on female reluctance (reluctance to mate is stereotypical and violent shaking behaviour). The proportion of females showing no reluctance to mate is displayed to facilitate comparison with Fig. 1. Error bars indicate ± 1 standard error.

importance of sexual conflict as a potential driver of speciation. Our results also contradict classical views of speciation[1], as divergence was faster in large (rather than small) populations. This result seems especially sound because potential inbreeding differences between populations should lead to lower mating rates in monogamous or low-density populations[14–16]. Notably, even if inbreeding had any influence, this would only alter conclusions concerning the effect of treatment on copulation frequency. The differences between matings within and between populations and the greater precopulatory reproductive isolation between high- than between low-density populations are unaffected. These cannot be explained by differential inbreeding alone, and the main conclusions would therefore remain unchanged. This seems to be especially true because low-density and monogamous populations have very similar population sizes and yet they differ markedly. Evidence of female (but not male) resistance to flies from other conflict populations implies that sexual conflict drove divergence. This asymmetry in responses is unlikely to be driven by differential natural selection. Additionally, divergent natural selection is not expected to cause populations under identical selection necessarily to diverge[17], but the pattern we see here is an explicit prediction of divergence through sexual conflict[2]. Nevertheless, other forms of sexual selection (for example, female choice) could potentially generate these results[18]. However, considering what is known of *S. cynipsea*[19–22], the pattern detected seems best explained by sexual conflict.

We have shown that increased population density/size increases the frequency of sexual interactions and thus sexual conflict. This increased sexual conflict caused a rapid increase in behavioural reproductive isolation, especially in larger, high-density populations, confirming the importance of conflict as a driving force in evolution. This provides experimental evidence for this new concept[2], which contrasts with classical population-genetics theory. □

Methods

Mating behaviour

Sepsis cynipsea is ideally suited for testing models of speciation by means of antagonistic processes because sexual conflict is blatantly obvious, with long and violent precopulatory struggles[19–22]. Conflict over copulation is due to injuries that males inflict on females during copulation, which reduce female fitness[22]. Males appear to largely control copulation duration[23], and longer copulations further reduce female fitness[24]. Previous work has also shown that female mating rates increase with the number of males present[25], and that females are more likely to re-mate with different males[26]. Additionally, copulation greatly increases female mortality[22].

Density and frequency of sexual interactions

We ensured that increased population size/density indeed led to increased sexual activity, as required by theory. Assays of the effects of population density on the frequency of sexual interactions were performed with unselected flies. Virgin one-week-old males were placed together with females in containers (standard size) with differing population densities (one container each with either 1, 5, 10, 25, 50 or 100 individual(s) of each sex). Over a period of 8 h the population cages were checked every hour and the number of copulating pairs and females exhibiting reluctance behaviour (vigorous shaking) were counted. From this the frequency of copulations, female reluctance behaviour and total sexual interactions were calculated. We found that density/fly number significantly increased the frequency of copulations and sexual interactions (repeated measures analysis of variance (ANOVA; controlling for number of flies present) indicated significant effects of density on the number of copulations ($F_{5,20} = 7.18$, $P = 0.0005$) and sexual interactions ($F_{5,20} = 5.04$, $P = 0.004$)). In total, five assays (including the six different densities in parallel) were performed. These findings indicate that at higher population densities, polyandry and hence sexual conflict increased. Additionally, housing females with males after a first copulation also lowers female fitness (lifetime offspring production: single copulated female housed alone = 78.8 ± 13.0; single copulated females housed with three males = 27.0 ± 6.4; analysis of co-variance (ANCOVA), $F_{1,37} = 11.4$, $P = 0.002$). This, in addition to previous data[27], shows that increasing the number of males that a female interacts with reduces her fitness, consistent with increased sexual conflict.

Selection lines

Stock populations were founded using male and female *S. cynipsea* collected at Fehraltorf in 2000 and maintained in population cages for five generations to allow acclimatization to laboratory conditions. Selection lines were started simultaneously including three treatment types each with three replicate populations. These consisted of high-density (HD1, 2 and 3, each with 250 males and 250 females per container), low-density (LD1, 2 and 3, each with 25 males and 25 females per container) and monogamous populations (M1, M2, M3, each consisting of 20 pairs in individual vials). High-density and low-density populations were maintained in large containers (same size for both treatments). For the monogamous populations, emerging offspring were sexed and randomly paired in 100-ml vials. The populations of all three treatments were given sugar, pollen and water *ad libitum* and supplied with fresh dung in small quantities every 3 days. However, the numbers of food, water and dung containers were varied so that they remained approximately proportional to the number of flies present. Importantly, by standardizing rearing conditions (food, temperature, relative humidity and photoperiod) across treatments, differential natural selection was not expected. Natural selection through density differences should be reflected in body size changes, as fly size responds rapidly to food or dung shortages[28]. However, we found no significant differences in body size across our selection regimes (female size: $F_{2,6} = 0.92$, $P = 0.45$; male size: $F_{2,6} = 0.22$, $P = 0.80$).

Flies were left to interact, and after approximately 12 days (30–60% of average laboratory lifespan) larger portions of dung were provided. These dung portions were collected and pooled per population and emerging flies were separated by sex. Flies for the next generation were taken randomly from these pooled offspring, which were always produced in larger numbers than required for maintaining populations. This allowed differential reproduction and ensured that the most successful genotypes per treatment (those which produce more offspring) were represented to a greater degree in the subsequent generation. We also synchronized the starting times of each generation between treatments to avoid differential selection on developmental times. All flies were maintained in the same large climate chamber (23–27 °C, 60–70% relative humidity, 12-h light/dark photoperiod), and cage and vial placement changed regularly to avoid microclimate effects.

Experiments

We investigated the effects of sexual conflict on population divergence in replicate laboratory *S. cynipsea* populations. After 35 generations of selection (different levels of conflict), flies were housed under relaxed selection for two generations to eliminate differential maternal effects and potential phenotypic effects generated by population density variation (all populations from all treatments in containers with 50 males and 50 females and kept for 12 days per generation as in the selection populations). The offspring of these flies were used for the experiments. To investigate population divergence, experimental females were placed separately with single males from the same treatment but from their own (within) or different populations (between) for 30 min and mating behaviour was assessed by direct observation. The numbers of females to copulate (only copulations with normal duration and uninterrupted genital contact) or show reluctance behaviour (vigorous shaking) were counted. In subsequent analyses, cross type (monogamous × monogamous, low density × low density or high density × high density) was the unit of replication and consisted of pairings between populations or within populations. For each cross type the behaviour of 50 females was observed (for example, 50 M1 females with M1 males for pairings within the M1 population or 25 M1 females with 25 M2 males plus 25 M2 females with 25 M1 males for pairings between populations M1 and M2, and so on).

Data analysis

Data were analysed using a general linear model. Before analysis, proportion data were arc-sin square-root-transformed. Residuals from all analyses did not differ significantly from a normal distribution (Kolmogorov–Smirnov tests, all $P > 0.05$).

Received 21 March; accepted 22 April 2003; doi:10.1038/nature01752.

1. Lande, R. Models of speciation by sexual selection on polygenic characters. *Proc. Natl Acad. Sci. USA* **78,** 3721–3725 (1981).
2. Gavrilets, S. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403,** 886–889 (2000).
3. Tregenza, T., Butlin, R. K. & Wedell, N. Sexual conflict and speciation. *Nature* **407,** 149–150 (2000).
4. Rice, W. R. & Hostert, E. E. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* **47,** 1637–1653 (1993).
5. Price, T. Sexual selection and natural selection in bird speciation. *Phil. Trans. R. Soc. Lond. B* **353,** 251–260 (1998).
6. Parker, G. A. in *Sexual Selection and Reproductive Competition in Insects* (eds Blum, M. S. & Blum, N. B.) 123–166 (Academic, New York, 1979).
7. Rice, W. R. & Holland, B. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav. Ecol. Sociobiol.* **41,** 1–10 (1997).
8. Swanson, W. J. & Vacquier, V. D. The rapid evolution of reproductive proteins. *Nature Rev. Genet.* **3,** 137–144 (2002).
9. Rice, W. R. in *Endless Forms: Species and Speciation* (eds Howard, D. J. & Berlocher, S. H.) 261–270 (Oxford Univ. Press, New York, 1998).
10. Schluter, D. *The Ecology of Adaptive Radiation* (Oxford Univ. Press, Oxford, 2000).
11. Rice, W. R. Dangerous liaisons. *Proc. Natl Acad. Sci. USA* **98,** 12953–12955 (2000).
12. Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. Sexual conflict promotes speciation in insects. *Proc. Natl Acad. Sci. USA* **97,** 10460–10464 (2000).
13. Gage, M. J. G., Parker, G. A., Nylin, S. & Wiklund, C. Sexual selection and speciation in mammals, butterflies and spiders. *Proc. R. Soc. Lond. B* **269,** 2309–2316 (2002).
14. Sharp, P. M. The effect of inbreeding on competitive male-mating ability in *Drosophila melanogaster*. *Genetics* **106,** 601–612 (1984).
15. Meffert, L. M. & Bryant, E. H. Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. *Evolution* **45,** 293–306 (1991).
16. Meffert, L. M. & Bryant, E. H. Divergent ambulatory and grooming behavior in serially bottlenecked lines of the housefly. *Evolution* **46,** 1399–1407 (1992).
17. Cooper, T. F., Rozen, D. E. & Lenski, R. E. Parallel changes in gene expression after 20,000 generations of evolution in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **100,** 1072–1077 (2003).

18. Andrés, J. A. & Morrow, E. H. The origin of interlocus sexual conflict: is sex linkage important? *J. Evol. Biol.* **16,** 219–223 (2003).
19. Parker, G. A. Reproductive behaviour of *Sepsis cynipsea* (L.) (Diptera: Sepsidae) I. A preliminary analysis of the reproductive strategy and its associated behaviour patterns. *Behaviour* **41,** 172–206 (1972).
20. Parker, G. A. Reproductive behaviour of *Sepsis cynipsea* (L.) (Diptera: Sepsidae) II. The significance of the precopulatory passive phase and emigration. *Behaviour* **41,** 242–250 (1972).
21. Ward, P. I., Hemmi, J. & Röösli, T. Sexual conflict in the dung fly *Sepsis cynipsea*. *Funct. Ecol.* **6,** 649–653 (1992).
22. Blanckenhorn, W. U. *et al.* The costs of copulating in the dung fly *Sepsis cynipsea*. *Behav. Ecol.* **13,** 353–358 (2002).
23. Martin, O. Y. & Hosken, D. J. Strategic ejaculation in the common dung fly *Sepsis cynipsea*. *Anim. Behav.* **63,** 541–546 (2002).
24. Martin, O. Y., Leugger, R. R., Zeltner, N. & Hosken, D. J. Male age, mating probability and mating costs in the fly *Sepsis cynipsea*. *Evol. Ecol. Res.* **5,** 119–129 (2003).
25. Blanckenhorn, W. U., Mühlhäuser, C., Morf, C., Reusch, T. & Reuter, M. Female choice, female reluctance to mate and sexual selection on body size in the dung fly *Sepsis cynipsea*. *Ethology* **106,** 577–593 (2000).
26. Hosken, D. J., Martin, O. Y., Born, J. & Huber, F. Sexual conflict in *Sepsis cynipsea*: female reluctance, fertility and mate choice. *J. Evol. Biol.* **16,** 485–490 (2003).
27. Mühlhäuser, C. & Blanckenhorn, W. U. The costs of avoiding matings in the dung fly *Sepsis cynipsea*. *Behav. Ecol.* **13,** 359–365 (2002).
28. Ding, A. & Blanckenhorn, W. U. The effect of sexual size dimorphism on mating behaviour in two dung flies with contrasting dimorphism. *Evol. Ecol. Res.* **4,** 259–273 (2002).

Acknowledgements We thank the SNF for financial support, and R. Leugger, K. Landergott and M. Eger for laboratory help. We also thank W. Blanckenhorn for statistical advice, F. Balloux for comments on the experimental design, and G. Arnqvist, A. Badyaev, F. Balloux, P. Bauerfeind, J. Evans, T. Garner, B. Holland, L. Keller, T. Pizzari and L. Simmons for valuable comments on this manuscript.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to O.M. (olma@zoolmus.unizh.ch).

Opposing basal ganglia processes shape midbrain visuomotor activity bilaterally

Huai Jiang, Barry E. Stein & John G. McHaffie

Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157, USA

The manner in which the nervous system allocates limited motor resources when confronted with conflicting behavioural demands is a crucial issue in understanding how sensory information is transformed into adaptive motor responses. Understanding this selection process is of particular concern in current models of functions of the basal ganglia[1]. Here we report that the basal ganglia use simultaneous enhancing and suppressing processes synergistically to modulate sensory activity in the superior colliculi, which are bilaterally paired midbrain structures involved in the control of visual orientation behaviours[2]. These complementary processes presumably ensure accurate gaze shifts mediated by the superior colliculi despite the presence of potential distractors.

Each superior colliculus (SC) contains a map-like representation of contralateral visual space that is in register with a motor map that produces movements of the head and eyes (or gaze shifts) to targets within that region of visual space[3]. Thus, the right SC controls gaze shifts to the left visual space, whereas the left SC effects movements to the right visual space. The specific vector of a gaze shift depends on the topographical locus of neural activity that the visual target induces within the contralateral SC[3]. This 'retinal error' signal is conveyed to brainstem regions that ultimately produce a gaze shift that brings the central retinae to bear on the target[4]. The basal ganglia are intimately involved in these SC-mediated processes through output neurons in the substantia nigra, pars reticulata (SNr)[5–8]. Two populations of nigrocollicular neurons are present anatomically[9,10], a robust uncrossed projection and a smaller crossed component.

Uncrossed neurons have high rates of spontaneous activity[5–8], and because they use the inhibitory neurotransmitter GABA (γ-aminobutyric acid)[11], activity in the SC output neurons that they contact[12,13] is normally suppressed[5]. However, before a gaze shift to a target in, for example, the right visual space, there is a temporary cessation of activity in a subset of uncrossed nigrocollicular neurons in the left SNr[7,8]. This phasically releases a corresponding subset of ipsilateral SC output neurons from inhibition and facilitates their activity; it is then conveyed to the contralateral brainstem and spinal cord[7,8]. These regions, in turn, activate the motor nuclei innervating the extraocular and neck muscles necessary to shift gaze to the desired location in space. This process of 'disinhibition' functions not only as the basic template by which the basal ganglia influence SC-mediated visuomotor behaviours, but also as the mode by which they interact with other motor-related structures[14]. However, nothing is known about the physiology of the crossed nigrocollicular neurons. Here we report that these neurons have unique physiological properties, and we suggest a potential role for the basal ganglia in the interhemispheric coordination of SC activity related to visuomotor behaviours.

Single-unit extracellular recordings were made from visually responsive neurons ($n = 303$) in the left SNr of anaesthetized cats. Of these neurons, 59 were antidromically activated from one or the other SC and were selected for quantitative analysis. They fell into two populations: one antidromically activated from the left SC and thus defined as uncrossed ($n = 43$; 73%) and another ($n = 16$; 27%) antidromically activated from the right SC and defined as crossed. No SNr neurons were encountered that could be antidromically activated from both SCs, indicating that these are segregated populations.

The two neuronal populations had different spatial distributions. All crossed neurons were recorded in the anterolateral aspect of SNr, at stereotaxic loci (anterior to posterior, +5.2–6.3 mm) where lesions have been shown to ameliorate cortically induced visual hemineglect[15]. In contrast, uncrossed nigrocollicular neurons were distributed over a broad rostrocaudal extent of SNr, which is consistent with anatomical reports[9]. The two neuronal populations also differed in conduction velocities. Assuming an average conduction distance of 7 and 13 mm, respectively, the conduction velocities of the uncrossed neurons were significantly lower (5.38 ± 2.036 (s.d.) m s^{-1}; range 1.98–10.4 m s^{-1}; $P < 0.01$; two-tailed t-test) than those of crossed neurons (mean 8.98 ± 3.699 m s^{-1}; range 3.64–14.2 m s^{-1}).

Despite these differences, all ($n = 59/59$; 100%) were orthodromically activated from regions of the ipsilateral lateral suprasylvian cortex, indicating that visual cortical activity can influence the SC by means of transynaptic corticofugal pathways that course through the basal ganglia. The patterns of activation observed in crossed and uncrossed neurons after orthodromic stimulation of extraprimary visual cortex were similar to those observed after electrical stimulation of frontal and motor cortex[16].

Uncrossed neurons displayed high rates of spontaneous activity (36.81 ± 18.08 Hz; range 11.06–86.26 Hz), consistent with their tonic inhibition of ipsilateral SC neurons[7,8]. Their visual receptive fields were relatively small (876 ± 443.11 deg^2; range 340–5,225 deg^2), with centres confined within the contralateral hemifield. There was also good spatial correspondence between their receptive fields and those of neurons at effective antidromic stimulation sites in the ipsilateral SC. Both had their receptive fields centred in contralateral space and in topographic register with each other ($n = 43/43$; 100%). All uncrossed neurons encountered

