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10 ml of saline, vortexing the mixture for several seconds, and then following standard selective plating protocol. The second plate was used for propagating the community (that is, for pressing on the velvet after the next day's growth). The third plate was photographed (see Fig. 3).

The Mixed Plate environment was identical to the Static Plate environment described above with the following exception: at each transfer, the fully-grown plate was (1) pressed lightly on the velvet, (2) turned clockwise at a randomly chosen angle and pressed a second time, (3) turned randomly counter-clockwise and pressed a third time, and (4) turned randomly clockwise and pressed a fourth time. The fresh plates were then pressed on the velvet to initiate this 'mixed up' sample.

Our Flask environment was a 125 ml flask with 33.75 ml of LB broth shaken at 125 rev min^{-1} at 37 °C. After 24 h of growth, 50 μl of the culture was transferred to a flask with fresh media. These volumes guaranteed that the total number of bacterial cells in the flask after a full day's growth, and total number of cells transferred, matched the corresponding numbers of cells from the plate runs.

In all three environments, the densities of each type were determined every 24 h, and all treatments were replicated in triplicate. We terminated the experiments after one week (approximately 66 generations), because by this time we detected a substantial number of resistant mutants derived from the S population (for example, the dense clumps within the C patches in Fig. 3a). Evolution of R from S violates an assumption of our model and can lead to a change in the predicted dynamics, especially if the cost of resistance is much lower in the *de novo* R mutants (see low values of Δ_R in Fig. 1e). We are currently exploring such evolutionary phenomena both theoretically and experimentally. It should be noted that one week suffices for a single 15-μl droplet placed in the centre of a plate to nearly cover the plate under the Static Plate regime.

Additional starting conditions

We manipulated the starting strain frequencies and initial spatial configuration on the plates in two additional sets of runs (data not shown). First, we repeated the experiments as outlined above with the same initial hexagonal lattice pattern, but using another random assignment of the strains (taken from the probability distribution above). Second, rather than initiating the plates with droplets in a hexagonal pattern, we mixed the bacterial strains in soft agar at different starting frequencies (with S in excess and C and R rare) and poured the agar over the plates, resulting in an initially 'unclumped' distribution.

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Exceptional sperm cooperation in the wood mouse

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Spermatozoa from a single male will compete for fertilization of ova with spermatozoa from another male when present in the female reproductive tract at the same time[1]. Close genetic relatedness predisposes individuals towards altruism, and as haploid germ cells of an ejaculate will have genotypic similarity of 50%, it is predicted that spermatozoa may display cooperation and altruism to gain an advantage when inter-male sperm competition is intense[2]. We report here the probable altruistic behaviour of spermatozoa in an eutherian mammal. Spermatozoa of the common wood mouse, *Apodemus sylvaticus*, displayed a unique morphological transformation resulting in cooperation in distinctive aggregations or 'trains' of hundreds or thousands of cells, which significantly increased sperm progressive motility. Eventual dispersal of sperm trains was associated with most of the spermatozoa undergoing a premature acrosome reaction. Cells undergoing an acrosome reaction in aggregations remote from the egg are altruistic in that they help sperm transport to the egg but compromise their own fertilizing ability.

Several examples of sperm cooperation have been reported mainly in molluscs and insects[3,4]. A possible exception in Mammalia is the spermatozoa of opossums that conjugate to form pairs during sperm maturation and disengage immediately before fertilization[5]. Sperm will benefit from cooperation if Hamilton's rule[6] is fulfilled. This depends on the probability of sperm survival in terms of

reaching the site of fertilization and the difference in relatedness of cooperating sperm and other sperm competing for fertilization. For true altruism, the fertilizing capacity of one spermatozoon is compromised or sacrificed to benefit another; however, evidence in Eutheria has been largely lacking. Spermatozoa of some rodents (for example, guinea-pig) stack in rouleaux formation[7] or agglutinate, but these cell associations do not appear particularly advantageous. Conversely, it has been suggested that a primary function of some spermatozoa in the rat and human ejaculate is to incapacitate spermatozoa of another male, so-called kamikaze spermatozoa[8,9]; however, this hypothesis is not supported by experimental evidence[10].

Among small mammals, multiple matings resulting in sperm competition and mixed paternity in littermates are believed to be widespread[1]. The wood mouse, *A. sylvaticus*, is a common murid rodent throughout Western Europe, with a breeding season from February to October[11]. Adult males were caught in woodland near Sheffield between February and September, or obtained from a breeding colony in captivity (Department of Veterinary Science, University of Liverpool). The weight of wild-caught mice (21.4 ± 2.9 g, $n = 10$) was not significantly different to that of males bred in captivity (22.6 ± 2.1 g, $n = 8$). Mean testes weight of 1.02 ± 0.16 g gave a relative testes/body mass of $4.76 \pm 0.38\%$, higher than for almost all rodent species and in accordance with high testis sperm output[12]. The cauda epididymidis lies underneath a hairless protrusion of the scrotum, a morphological adaptation that may have evolved for efficient cooling and storage of spermatozoa, thereby maximizing ejaculate sperm output[13]. These features are consistent with a high degree of inter-male sperm competition[1,14], as also indicated by a radio-tracking study that concluded that male wood mice engage in scramble competition to mate polygynously with promiscuous females[15]. This suggests that sperm competition and multiple paternity is probably commonplace in wood mice; however, as far as we are aware, direct DNA parentage studies have not been undertaken.

The murid rodent sperm head usually displays a falciform morphology[16]. In the wood mouse, this morphology developed after meiosis and was complete at spermiation (Fig. 1a). Epididymal spermatozoa exhibited an extremely long apical hook composed of an extended perforatorium region[17] (Fig. 1b, c). Uniquely, the hook was attached invariably (>95%) to a peri-nuclear process, spur and neck region on the lower ventral surface of the sperm head by electron-dense adhesive material. Propidium iodide staining indicated that only the basal region of the apical hook was of nuclear origin (Fig. 1c, d). The acrosome was situated over the convex and upper lateral region of the sperm head but extended for only part of

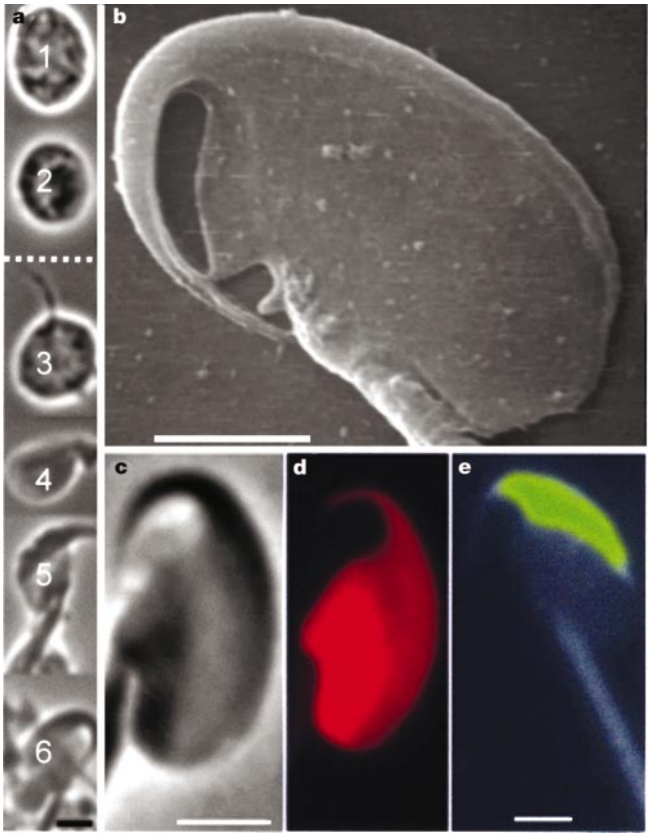

Figure 1 Morphology of wood mouse sperm head. **a**, Montage of germ cells extracted from wood mouse testis. 1, spermatogonia; 2, pachytene spermatocyte. Below the dotted line indicates spermiogenesis: 3, round spermatid with flagellum; 4 and 5, elongating spermatid; 6, testicular spermatozoa at spermiation from epithelium, note completion of apical hook. **b**, Scanning electron micrograph of a sperm head showing attachment of apical hook to ventral shelf, stud and neck region. **c**, Phase-contrast image of sperm head. **d**, Propidium iodide nuclear staining of **c** showing the limit of nuclear contribution to the hook. **e**, Immunofluorescent staining of acrosomal region. Scale bars: **a**, 2 μm; **b**, 2.5 μm; **c**–**e**, 3 μm.

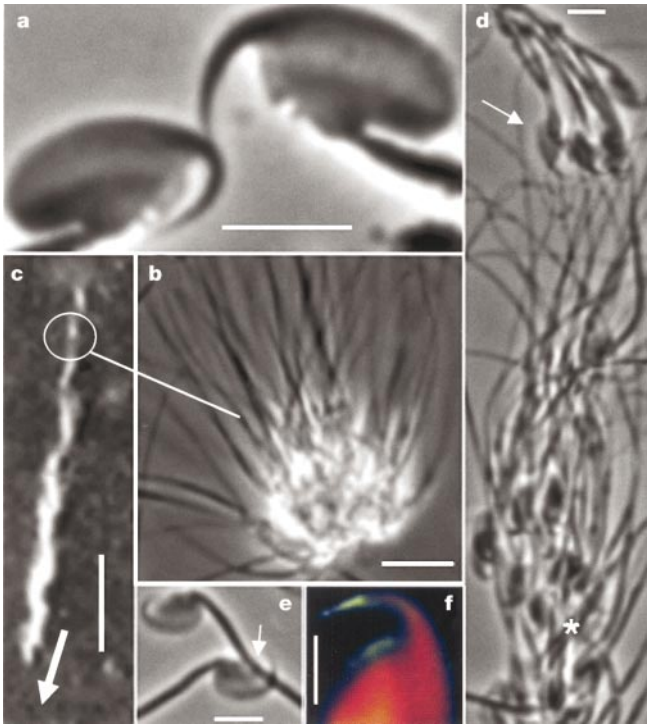

Figure 2 Development of sperm train. **a**, Phase-contrast image of spermatozoa after 2 min in IVF medium. The spermatozoon on the right has fully deployed its hook; compare with the spermatozoon on the left where the hook is still attached laterally to the head. **b**, Motile aggregation of approximately 50 spermatozoa. **c**, Dark-field frame from a video clip of a large motile sperm train over 2-mm in length and consisting of thousands of spermatozoa. In each smaller clump in the line (circled), spermatozoa were aggregated as in **b**. The train moved in the direction of the arrow with rapid sinusoidal motility. **d**, Phase-contrast image of formalin-fixed spermatozoa from a train. Sperm attach by means of apical hook to flagellum (arrow), or apical hook to apical hook (asterisk). **e**, Phase-contrast of a spermatozoon attached by the apical hook to the flagellum of another cell (indicated by an arrow). **f**, Fluorescent (fluorescein isothiocyanate) immunolocalization of filamentous actin in the sperm head with propidium iodide counterstain. Actin was expressed in the apical hook region more intensely after deployment. Scale bars: **a**, **d**, **e**, **f**, 5 μm; **b**, 10 μm; **c**, 1 mm.

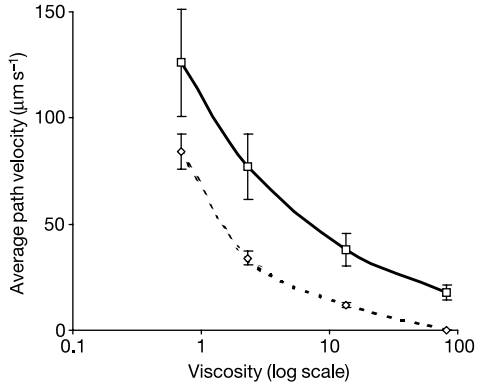

Figure 3 The change in progressive motility (average path velocity) of sperm trains (solid line) and single spermatozoa (dotted line) with increasing viscosity of medium. Sperm trains displayed significantly greater average path velocity ($P \leq 0.01$) than single spermatozoa at all values.

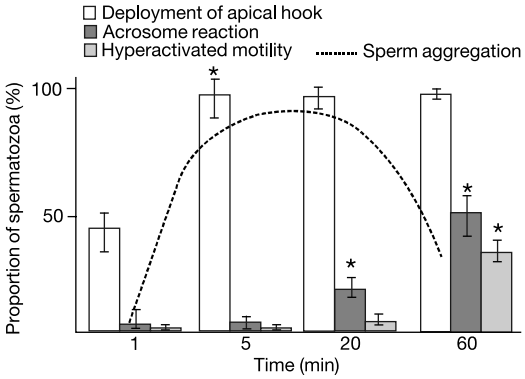

Figure 4 Histogram of the proportion of spermatozoa displaying deployment of the apical hook, the acrosome reaction and hyperactivated motility in relation to sperm aggregation *in vitro* at different time points. Error bars indicate s.e.m. Asterisk, significantly different from previous time point ($P \leq 0.005$; $n = 6$).

the hook, as visualized with acrosome-specific monoclonal antibody 18.6 (ref. 18) (Fig. 1e).

Cauda epididymal spermatozoa displayed good motility (≥90% progressively motile) when released into laboratory mouse *in vitro* fertilization medium[19] and initially were in single cell suspension. Within 1–5 min, the apical hook of each spermatozoon moved away from the ventral process and spur (Fig. 2a) and intertwined and adhered to other deployed apical hooks or to the flagellum of other spermatozoa, forming motile clumps of 10–50 cells (Fig. 2b). By 5 min most of these clumps (≥85%) formed motile 'trains' of spermatozoa comprising hundreds to several thousand cells (Fig. 2c, d). These trains exhibited rapid progressive motility, often with a sinusoidal motion (see Supplementary Information). As determined by computer-assisted sperm analysis[20], the mean average path velocity of sperm trains ($132 \pm 21\,\mu m\,s^{-1}$, $n = 200$ per sample) was significantly greater than for single spermatozoa ($87 \pm 24\,\mu m\,s^{-1}$, $P \leq 0.05$). Initiation of sperm aggregations was concomitant with adhesion of the inner concave surface of the apical hook with flagellum (Fig. 2e) or apical hooks of other spermatozoa. The intensity of staining for filamentous actin (but not other cytoskeletal proteins; data not shown) in the apical hook increased after its deployment, suggesting that this protein might have a function in hook remodelling (Fig. 2f). Cell–cell adhesion between spermatozoa occurred along the inner surface of the apical hook. The difference in progressive motility between single spermatozoa and trains was relatively more pronounced when the viscosity of medium was increased with polyvinyl pyrrolidone (PVP-10) to that estimated for secretions of the female reproductive tract[5] (Fig. 3). At the highest viscosity (10% PVP-10), progressive motility was exhibited only by sperm trains. Several reports indicate that the mean progressive motility of spermatozoa, as measured *in vitro*, is positively correlated with the outcome of fertilization *in vivo*[20–22].

To eliminate the possibility that sperm trains resulted from an artefact of *in vitro* culture conditions, sperm behaviour in the female tract was investigated after mating. Captive-bred adults were maintained in breeding groups of typically four females and two males (14 h light/10 h dark cycle, dusk at 16:00). After mating (0.5–2 h), females ($n = 5$) were killed and their reproductive tract removed and flushed with phosphate buffered saline by fine pipette under a dissecting microscope. Motile sperm trains (approximately 50–200 sperm) and many non-motile single spermatozoa were recovered from the vagina of each female. In three females, motile sperm trains (10–50 sperm) were detected in the uterine lumen but very few (<5%) single spermatozoa.

Dispersal of the 'train' of spermatozoa occurred after about 30 min and was complete by 90 min in capacitating medium. Spermatozoa retained motility (≥90%) but there was a significant increase in the proportion of spermatozoa that had undergone premature loss of the acrosome (as detected by monoclonal antibody 18.6) and showed hyperactivated motility. A time course for these changes in relation to deployment of the apical hook and sperm aggregation is shown in Fig. 4.

Such close cooperation of many spermatozoa to significantly enhance sperm motility is rare and thus far has not been observed in this manner in any mammalian species. We hypothesize that intermale sperm competition in conjunction with unique morphological features of the sperm head were conducive for evolution of this elaborate gamete behaviour. In the male excurrent ducts, where spermatozoa are densely packed, an exposed elongated apical hook is liable to cause sperm aggregation and blockage, leading to infertility. An adaptation where the hook is initially attached to the lower ventral surface of the sperm head would prevent premature sperm aggregation but subsequent deployment of the hook in the female reproductive tract would promote beneficial sperm aggregation and sperm motility. A 'green beard' gene might evoke such cellular cooperation although other genetic mechanisms cannot be excluded at this stage. A green beard is a gene that recognizes copies of itself in other individuals and directs benefits to those individuals[23]. Such genes (or groups of linked genes) are predicted to be rare because they must code for a combination of features including a recognizable morphological or behavioural phenotype, a recognition system and a response (in this case enhanced sperm motility particularly in viscous secretion)[24]. During mammalian spermatogenesis, germ cells proliferate and differentiate while remaining connected by inter-cytoplasmic bridges, allowing gene products to diffuse freely between individual cells[25]. In such a syncytium, most products of post-meiotic gene expression will tend to be equilibrated between spermatozoa, particularly membrane surface components. Thus, it may be significant that the mechanism in *A. sylvaticus* entails a non-diffusible component, namely the perforatorium, and therefore a modification to a trait could remain specific to the cell. The perforatorium and apical hook clearly developed and attached to the lower ventral sperm head during post-meiotic testis development in the wood mouse. Although some genes involved in the formation of these organelles may be first expressed at the diploid stage, many others such as testis-specific gamma actin have only been detected in post-meiotic germ cells[26,27]. Single mutations in spermiogenic-specific genes can profoundly affect sperm-head morphology[28].

A mechanism for dispersal of aggregated sperm before fertiliza-

tion is required. This was concomitant with induction of the acrosome reaction in a proportion of the aggregated spermatozoa *in vitro*, and acrosomal proteolytic enzymes may be responsible for digestion of cell–cell adhesion molecules. Acrosome-reacted murine spermatozoa lose their capacity to bind to the zona pellucida and fertilize[29], and therefore these cells displayed altruistic behaviour. In contrast, acrosome-intact spermatozoa free of aggregation would retain fertilizing capacity[29]. □

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Transmitter-evoked local calcium release stabilizes developing dendrites

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In the central nervous system, dendritic arborizations of neurons undergo dynamic structural remodelling during development. Processes are elaborated, maintained or eliminated to attain the adult pattern of synaptic connections[1–3]. Although neuronal activity influences this remodelling[4–6], it is not known how activity exerts its effects. Here we show that neurotransmission-evoked calcium (Ca^{2+}) release from intracellular stores stabilizes dendrites during the period of synapse formation. Using a ballistic labelling method to load cells with Ca^{2+} indicator dyes[7], we simultaneously monitored dendritic activity and structure in the intact retina. Two distinct patterns of spontaneous Ca^{2+} increases occurred in developing retinal ganglion cells—global increases throughout the arborization, and local 'flashes' of activity restricted to small dendritic segments. Blockade of local, but not global, activity caused rapid retraction of dendrites. This retraction was prevented locally by focal uncaging of caged Ca^{2+} that triggered Ca^{2+} release from internal stores. Thus, local Ca^{2+} release is a mechanism by which afferent activity can selectively and differentially regulate dendritic structure across the developing arborization.

To observe directly how neural activity dynamically regulates dendritic structure during development, we introduced Ca^{2+} indicator dyes into retinal ganglion cells by a ballistic method that resulted in complete filling of the dendritic arborization[7] (Fig. 1a). Using Oregon Green 488 BAPTA-1 dextran (OGB1; $k_d = 0.3$–$0.4\,\mu M$), Ca^{2+} levels were monitored across the dendritic arborization of embryonic days (E) 8–19 chick retinal ganglion cells ($n = 93$ cells), during the period of dendritic remodelling and synaptogenesis. Two distinct types of Ca^{2+} transients were observed (Fig. 1b and c; see Supplementary Information 1). First, local patches of dendrites showed transient increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) that extended from 5.0 to 16.0 μm (mean ± s.e.m. = 9.6 ± 0.9 μm; 5 cells, 12 events) and lasted between 3.0 and 28.0 s (8.9 ± 2.5 s; 7 cells, 26 events). The mean change in fluorescence ($\Delta F/F_0$; see Methods) of these events was 27.0 ± 1.4% (12 cells, 129 events). With a lower affinity indicator, Fluo-4 dextran ($k_d = 4.1\,\mu M$, $n = 3$ cells, 5 events; see Supplementary Information 2A), we observed local events with similar duration (10.2 ± 1.8 s) and extent (9.8 ± 2.9 μm), albeit at lower amplitude ($\Delta F/F_0 = 13.2 \pm 1.9\%$). Local flashes appeared with a frequency of $5.2 \pm 0.7\ min^{-1}$ per mm dendrite at E13. Qualitative comparison of the onset times of local events across the arborization did not reveal any strict temporal patterns in their occurrence, although they can occur repeatedly in the same locations (Fig. 1e).

In addition to local flashes, we observed Ca^{2+} levels simultaneously increasing in the soma and throughout the arborization (Fig. 1b and c). Global activity was periodic at intervals of 80.0 ± 16.0 s, occurring synchronously in neighbouring cells. In contrast to local flashes, global activity was shorter in duration (2.0–7.0 s, mean 3.9 ± 0.7 s, 4 cells, 10 events) and relatively larger in amplitude ($\Delta F/F_0$: 53.0 ± 4.8%, 12 cells, 23 events). The global activity resembled that of spontaneous bursting activity observed during propagation of retinal waves[8–10]. Simultaneous calcium imaging and perforated patch recordings (5 cells) demonstrated