THE RAPID EVOLUTION OF REPRODUCTIVE PROTEINS

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Many genes that mediate sexual reproduction, such as those involved in gamete recognition, diverge rapidly, often as a result of adaptive evolution. This widespread phenomenon might have important consequences, such as the establishment of barriers to fertilization that might lead to speciation. Sequence comparisons and functional studies are beginning to show the extent to which the rapid divergence of reproductive proteins is involved in the speciation process.

ADAPTIVE EVOLUTION
A genetic change that results in increased fitness.

ORTHOLOGOUS GENES
Homologous genes in different species that derive from a common ancestral gene without gene duplication or horizontal transmission.

PURIFYING SELECTION
Selection against a deleterious allele.

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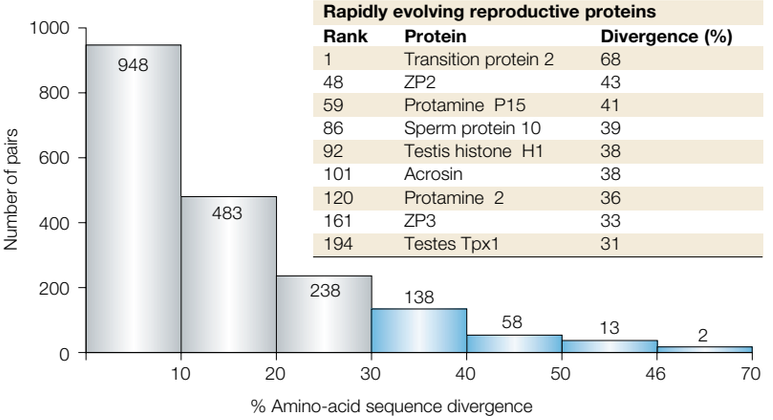
Comparing gene sequences within and between closely related species has shown that the genes that mediate sexual reproduction are more divergent than the genes that are expressed in non-reproductive tissues[1,2]. For example, using two-dimensional electrophoresis, Civetta and Singh[3] have shown that proteins from reproductive tissues in *Drosophila* are twice as diverse as proteins from non-reproductive tissues. In many cases, this rapid divergence is driven by ADAPTIVE EVOLUTION (positive Darwinian selection)[4], which indicates that sequence diversification is beneficial to reproduction. This emerging generalization might be important for our understanding of how speciation occurs once populations have become reproductively isolated. In this review, we focus on reproductive proteins that are evolving rapidly. We broadly define reproductive proteins as those that act after copulation and that mediate gamete usage, storage, signal transduction and fertilization. We review work showing that the rapid evolution of reproductive proteins occurs in several taxonomic groups and present possible causes for their rapid evolution. One important remaining issue is to understand the functional consequence of rapidly evolving reproductive proteins. We suggest that the co-evolution of corresponding (interacting) female and male pairs of such proteins could be a factor in the establishment of barriers to fertilization, which lead to reproductive isolation and the establishment of new species.

Rapid evolution

Rapidly evolving genes are those that encode proteins with a higher than average percentage of amino-acid substitutions between species. In one study, Makalowski and Boguski[5] compared 1,880 proteins that are encoded by ORTHOLOGOUS GENES from humans and rodents, which represent ~5% of all predicted human genes. Fifty per cent of them showed less than 10% divergence at the amino-acid level (FIG. 1), and 209 fell within the range of 30–70% divergence. Although many of these most rapidly evolving genes are involved in the immune response, eight are involved in reproduction. Three of them — *ZP2* (zona pellucida glycoprotein 2), *ZP3* and *ACR* (acrosin) — are directly involved in the sperm–egg interaction. The fact that proteins that are involved in such a crucial process as fertilization are not conserved poses an interesting question for evolutionary biologists: Why are reproductive genes evolving so rapidly, and what is the functional consequence of this rapid evolution?

Identifying rapidly evolving genes by total percentage divergence does not provide information about the potential causes of rapid evolution. For example, rapid evolution might be due to a lack of functional constraint; for example, a pseudogene might rapidly accumulate mutations because of an absence of PURIFYING SELECTION. Alternatively, rapid evolution might be due to adaptive evolution, which occurs when natural selection promotes amino-acid divergence. One way to distinguish between these two alternatives is to compare DNA sequences of the protein-coding regions between species. Each nucleotide change is then classified either as a non-synonymous change, which alters the amino-acid sequence, or a synonymous (silent) change, which does not change the amino-acid sequence[4,6]. Because the number of non-synonymous and synonymous sites in any protein-coding sequence is unequal, these



Rapidly evolving reproductive proteins

Rank	Protein	Divergence (%)
1	Transition protein 2	68
48	ZP2	43
59	Protamine P15	41
86	Sperm protein 10	39
92	Testis histone H1	38
101	Acrosin	38
120	Protamine 2	36
161	ZP3	33
194	Testes Tpx1	31

Figure 1 | **Rapidly evolving proteins.** Comparison of 1,880 human–rodent orthologues from Makalowski & Boguski[5] plotted as a frequency of the occurrence of genes with a varying percentage of amino-acid divergence. The portion that contains the 10% most divergent proteins is shown in blue; reproductive proteins that are among the 10% most divergent proteins are listed. Tpx1, testis-specific protein 1; ZP2/3, zona pellucida 2/3.

values are often normalized to the number of sites (nucleotide positions) in the coding region. d_N and d_S define the number of non-synonymous substitutions per possible non-synonymous codon sites and the number of synonymous substitutions per possible synonymous codon sites, respectively, and the two values can then be compared directly. A d_N/d_S ratio of 1 indicates neutral evolution, but a significantly higher ratio indicates positive Darwinian selection. A ratio significantly greater than 1, when $d_N > d_S$, can only be obtained by positive selection for amino-acid change[4]; by contrast, the average d_N/d_S ratio between 45 conserved mouse and human genes is 0.2 (REF. 6). The d_N and d_S values can be averaged across the entire gene[7], or estimated from predicted binding sites[8]. If sequences are available from several species, new MAXIMUM-LIKELIHOOD prediction methods can be used to detect selection that acts on a subset of codons[9]. Importantly, these new methods do not require *a priori* knowledge of the sites under selection and can be used to predict the functionally important sites of a gene that are subject to positive Darwinian selection[10]. A signal of positive Darwinian selection indicates that there is an adaptive advantage to changing the amino-acid sequence, and this signal can be used to identify functionally important gene regions, such as binding sites[10,11]. This is a different perspective from the typical way of identifying functionally important gene regions, which proceeds by looking for regions of conserved sequence.

Extensively diverged reproductive proteins

At present, the amino-acid sequences of only a few male and female pairs of reproductive proteins that bind each other to mediate fertilization are known. However, many eukaryotes have reproductive proteins that show extensive divergence between closely related species (TABLE 1). Below are a few examples of rapidly diverging reproductive proteins. Marine CILIATES of the genus *Euplotes* secrete protein pheromones of 40–43 amino acids that mediate sexual CONJUGATION and vegetative growth. An alignment of seven of these pheromone sequences from different mating types of one species shows that only seven amino acids have been conserved, six of which are cysteines that form three conserved disulphide bonds[12].

Two genes that control mating in the unicellular green alga *Chlamydomonas reinhardtii* are very divergent between species. The product of the *Chlamydomonas MID* gene determines if a cell will be of mating type + or –, whereas *FUS1* encodes a protein needed for fusion of + and – cells. However, no homologues of *FUS1*, and only one homologue of *MID*, in *C. reinhardtii* have been found in 12 other *Chlamydomonas* species[13,14].

An extracellular matrix protein encoded by the *Sig1* gene from the DIATOM *Thalassiosira* spp. is upregulated during mating and is thought to function in the mating process. *Sig1* is highly divergent both within and between species, and there are well-documented differences that distinguish between *Sig1* from the Atlantic and the Pacific Oceans[15]. Although the exact function of the Sig1 protein remains unknown, its extreme divergence indicates the possibility that it might be a barrier to reproduction between different diatom strains.

Mating compatibility in basidiomycete fungi requires secretion of protein pheromones that bind to cell-surface receptors and mediate signal transduction, which leads to the expression of mating genes[16]. The pheromones and their receptors show extreme sequence variation[17], which could underlie species-specific gamete interaction. Although reproductive genes from *Euplotes*, *Chlamydomonas*, *Thalassiosira* and basidiomycetes differ between species, there is no evidence at present that this divergence is promoted by positive Darwinian selection. Additional studies are needed to determine which selective pressures cause the rapid evolution of these genes. Once cDNA sequences for each of these genes are compared between several species, it will be possible to test whether positive Darwinian selection is promoting their divergence.

Many species of flowering plants cannot self-fertilize because their pollen is incompatible with stylar (female) tissue, a reproductive strategy that is thought to prevent INBREEDING DEPRESSION. In SPOROPHYTIC self-incompatibility in the genus *Brassica*, the pollen component is encoded by the highly variable *S*-locus cysteine-rich gene *SCR*[18]. The stylar recognition *S*-locus receptor kinase (*SRK*) encodes a membrane-spanning protein kinase and is also highly variable[19]. So, *SRK* and *SCR* comprise a pair of gamete-recognition proteins[20]. SCR is similar to the *Euplotes* pheromones in that, although the SIGNAL SEQUENCES of SCR proteins are relatively conserved, the mature SCR proteins only have nine out of about 50 identical amino-acid positions between seven alleles[21]. In GAMETOPHYTIC self-incompatibility in the *Solanaceae*, the pollen component has yet to be identified, but the stylar product of the self-incompatibility gene encodes an extracellular RNase encoded by the *S*-locus. *S*-alleles can differ by 50% in amino-acid identity within the same species[22] and show a clear signature of positive Darwinian selection by having a d_N/d_S ratio greater than 1 (REF. 22), which indicates that there is a reproductive benefit for sequence diversity at this locus. Components

MAXIMUM LIKELIHOOD
The maximum-likelihood method takes a model of sequence evolution (essentially a set of parameters that describe the pattern of substitutions) and searches for the combination of parameter values that gives the greatest probability of obtaining the observed sequences.

CILIATE
A single-celled protist with a micronucleus (germ-line nucleus), a macronucleus (somatic nucleus), and cilia for swimming and capturing food.

CONJUGATION
The joining of two cells for the transfer of genetic material.

DIATOM
A unicellular alga that is important in global photosynthesis and carbon cycling.

INBREEDING DEPRESSION
Loss of vigour owing to homozygosity of an increasing number of genes; it occurs as a consequence of mating between closely related individuals.

SPOROPHYTE
In plants that undergo alternation of generations, a multicellular diploid form that results from a union of haploid gametes and that meiotically produces haploid spores, which will in turn grow into the gametophyte generation.

Table 1 | **Rapidly evolving genes involved in fertilization**

Gene	Function	Organism	References
Pheromones (such as *Er1*)	Mating and cell growth	*Euplotes* (ciliate, protozoa)	12
mid1	Determines mating type + or –	*Chlamydomonas* (green alga)	13
fus1	Mediates cell fusion	*Chlamydomonas*	14
Sig1	Involved in cell mating	*Thalassiosira* (diatoms)	15
Pheromones (such as Phb.3.2)	Mating-type pheromone	Basidiomycetes (fungi)	17
SCR	Sporophytic self-incompatibility	Brassicaceae	18,19
S-locus	Gametophytic self-incompatibility	Solanaceae	22
Lysin	Dissolves egg envelope	*Tegula* and abalone (Mollusca)	24,26
sp18	Fusagenic sperm protein	Abalone	28
TMAP	Major acrosomal protein	*Tegula*	29
Bindin	Adheres sperm to egg	Sea urchin	34,35
Acp26Aa, Acp36DE	Sperm usage and storage	*Drosophila*	49,51,52
Ph-20, *β-fertilin*	Sperm-surface recognition	Mammals	54
ZP3	Egg inducer of sperm acrosome reaction	Mammals	10
ZP2	Egg envelope, sperm binding	Mammals	10
OGP	Oviductal glycoprotein	Mammals	10
Zonadhesin	Sperm surface	Mammals	59
TCTE1	Mammalian spermatogenesis	Mammals	60

Acp, accessory-gland proteins; *Er1*, *E. raikovi* pheromone type 1; *OGP*, oviductal glycoprotein; *SCR*, *S*-locus cysteine-rich; *TCTE1*, t-complex-associated-testis-expressed 1; *TMAP*, the major acrosomal protein; *ZP2/3*, zona pellucida 2/3.

of the pollen coat from *Arabidopsis thaliana* also show extensive variability[23].

Immediately before fertilization, sperm of marine gastropods of the genus *Haliotis* (abalone) and the genus *Tegula* (turbin snails) release a soluble protein, lysin, from the ACROSOME onto the surface of the egg envelope. In a species-specific, non-enzymatic process, lysin creates a hole in the egg envelope through which the sperm swim to reach the egg cell membrane. The amino-acid sequences of lysins from different species are extremely divergent and there is evidence that this divergence has come about through adaptive evolution[24–26]. Abalone sperm also release a protein (sp18) that is thought to mediate the fusion of the sperm and egg[27]. In five Californian abalone species, sp18 proteins are up to 73% different at the amino-acid sequence level[28] and there is evidence that this protein might evolve up to 50 times faster than the fastest evolving mammalian proteins[25]. A striking demonstration of this rapid evolution is seen when intron and exon divergence rates are compared between species — exons seem to evolve 20 times faster than the introns[25] (TABLE 2). In addition to lysin, *Tegula* sperm also release a major acrosomal protein of unknown function that is highly divergent and subject to adaptive evolution[29]. Abalone lysin and sp18 are perhaps the most robust examples of strong positive Darwinian selection that promotes amino-acid diversification. Although the driving force behind this rapid evolution is not yet clear, it has been suggested that the rapid diversification of sperm lysin is driven by its need to interact with a constantly changing egg receptor[30,31] (see below).

Rapid, extensive evolution of reproductive proteins has also been seen in sea urchins, the sperm of which use a protein called bindin to attach to the egg surface[32] and possibly to fuse with the egg cell membrane. *Echinometra mathaei* and *Echinometra oblonga* are two SYMPATRIC species that live in the Pacific, and on the basis of the comparisons of mtDNA sequences, they are the most closely related sea urchin species known. Because adhesion of bindin to eggs has evolved to be species specific[33], few inter-species hybrids are formed. Bindin sequences show remarkable divergence both within and between *Echinometra* species[34], as well as between species of another sea urchin genus, *Strongylocentrotus*[35]. In both *Echinometra* and *Strongylocentrotus* bindin[35], a region with an elevated d_N/d_S ratio has been identified as a target of positive selection. The exact function of this region remains unknown, but it might be involved in the species-specific adhesion of sperm to eggs.

Non-marine invertebrates also show rapid adaptive evolution of reproductive proteins, and the accessory gland proteins of *Drosophila* are the best-characterized example[36,37]. During copulation, an estimated 83 protein products of the *Drosophila* male accessory glands[11] are transferred along with sperm to the female reproductive tract[36]. These seminal fluid molecules increase the female's egg-laying rate[38–42], reduce her receptivity to further mating[38,39,42], promote sperm storage in the female[43–45], reduce her lifespan[46] and are involved in sperm competition[47,48]. (This topic will be discussed in more detail in the forthcoming special issue on the evolution of sex.) It has been shown that the accessory-gland proteins are twice as diverse between species as are non-reproductive proteins[3]. Although DNA analysis confirms this twofold increase in the rate of amino-acid replacement between species[11], the molecular evolution of only a few accessory gland proteins has been studied in detail. In particular, the gene that encodes the accessory gland protein Acp26Aa is one of the fastest evolving genes in the *Drosophila* genome, and a d_N/d_S ratio of 1.6 between *Drosophila melanogaster* and *Drosophila yakuba* indicates that its evolution is driven by positive Darwinian selection[49,50]. Other accessory-gland proteins that show signs of positive selection include Acp36DE (REF. 51) and Acp29AB (REF. 52). The divergence of accessory gland proteins has been shown to be partly responsible for species-specific usage of gametes in some *Drosophila* species[53]. For example, crosses between female *Drosophila suzukii* and male *Drosophila pulchrella* do not produce hybrid offspring, in spite of sperm transfer. However, hybrids between these two species are formed if, after being mated with *D. pulchrella* males, *D. suzukii* females are injected with accessory-gland extracts from *D. suzukii* males, which indicates that the presence of species-specific accessory-gland proteins is required for reproduction[53].

SIGNAL SEQUENCE
A short sequence on a newly translated polypeptide that serves as a signal for its transfer to the correct subcellular location.

GAMETOPHYTE
In a reproductive cycle of a plant, a generation that has a haploid set of chromosomes and produces gametes.

ACROSOME
A secretory organelle in the sperm head.

SYMPATRIC
Having overlapping geographical distributions.

Table 2 | **Percentage sequence difference* in three abalone species**

Gene	Percentage nucleotide difference		
	Hru–Hco	Hru–Hfu	Hco–Hfu
Lysin			
Exons (420 bp)	13.7	24.1	22.1
Introns (2187 bp)	3.0	4.8	5.8
sp18			
Exons (447 bp)	83.6	81.3	92.8
Introns (745 bp)	ND	5.1	ND

*All distances are Jukes–Cantor corrected for multiple substitutions from Metz *et al.*[25]. (As the time of divergence between two sequences increases, so does the probability that nucleotide substitutions occur that revert to the original sequence. For this reason, counting substitutions as a measure of divergence can be misleading and Jukes–Cantor correction helps to avoid the problem.) Intron values are the average for two or three introns, for *sp18* and *lysin*, respectively. Hco, *H. corrugata*; Hfu, *H. fulgens;* Hru, *H. rufescens*.

The rapid, adaptive evolution of reproductive proteins and species-specific fertilization is not limited to invertebrates, as similar phenomena have also been described in mammals[10,54]. Mammalian eggs are enclosed in an envelope called the zona pellucida (ZP), which is composed of three major glycoproteins — ZP1, ZP2 and ZP3 (REF. 55). ZP3, or a combination of ZP glycoproteins[56], is the first to bind the sperm to the ZP, and this binding is responsible for the species-specific induction of the acrosome reaction[55]. Analysis of ZP3 sequences from eight mammalian species indicates that two ZP3 regions, which directly participate in sperm binding[57,58], undergo rapid adaptive evolution[10]. One of these regions is specifically involved in the species-specific induction of the acrosome reaction[57], which indicates that the selective pressure for this protein to adapt relates to the sperm–egg interaction. ZP2, another rapidly evolving protein that is also subject to adaptive evolution[10], is involved in the tight binding of sperm to the ZP that occurs after the acrosome reaction[55].

Many sperm-surface proteins that bind to mammalian eggs have been isolated; for example, a mouse sperm-surface hyaluronidase (Ph-20, also known as Spam, sperm adhesion molecule), a protein that mediates adhesion of sperm to the egg plasma membrane (β-fertilin)[54] and proteins that are involved in binding sperm to the ZP (zonadhesin[59] and TCTE1 (t-complex-associated-testis-expressed 1) (REF. 60)). At present, there is no consensus on the identity and function of these proteins, but although it is clear that they evolve rapidly, so far there is no sign that adaptive evolution promotes the divergence of these mammalian reproductive proteins.

Species-specific fertilization

The sperm–egg interaction that leads to gamete fusion and zygote formation is most efficient if the sperm and the egg are from the same species. Even in very closely related species of sea urchins[33], fruitflies[61], nematodes[62] and mammals[63], strong barriers to cross-species fertilization have evolved. The phenomenon of species-specific fertilization shows that the proteins that are involved in gamete recognition must have a species-specific structure and that they must bind each other with species-specific affinity. So, a pair or a suite of fertilization proteins — that is, one or more male and female proteins — has to co-evolve to maintain their interaction. The inability of sperm to fertilize eggs creates a reproductive barrier that could subdivide populations into species. But how does species-specific fertilization evolve? And when does this evolution occur — does it happen at the early stages of species divergence, or do the changes accumulate only after speciation?

To find answers to some of these questions, evolutionary biologists have turned to one of the most extensively characterized animal fertilization systems, that of the abalone, because amino-acid sequences for lysin and its receptor, VERL, are known for several closely related species of the abalone. Once it is released from the acrosome, lysin binds to VERL molecules of the egg vitelline envelope in a species-specific manner[64,65]. The fibrous VERL molecules then lose their cohesion and splay apart, creating a hole through which the sperm swims[30,65]. Amino-acid sequences of lysins from different abalone species are remarkably divergent and are excellent examples of adaptive evolution[25,26]. The cause of rapid evolution of lysin might lie in the structure of VERL; it is a large, ~1,000 kDa glycoprotein that contains 22 tandem repeats of ~153 amino acids. In contrast to lysin, VERL shows no evidence of positive Darwinian selection; instead, it seems to be evolving neutrally. The 22 tandem VERL repeats are subject to concerted evolution — the mechanism by which ribosomal genes evolve[30,66], in which unequal crossing over and GENE CONVERSION randomly homogenize the sequence of tandem repeats within the gene and within a population[66,67]. The end result is that the repeats in a molecule from one species are more similar to each other than they are to homologous repeats in molecules from other species.

The study of the mechanisms of speciation is one of the central areas of interest in evolutionary biology. Although the role of rapid evolution of reproductive proteins in the speciation process is intriguing, undoubtedly there are many mechanisms by which animal populations could become reproductively isolated from each other and evolve into new species[68]. For example, hybridization can lead to the formation of new species in wild sunflowers of the genus *Helianthus*[69]. The question of how speciation occurs is especially interesting for marine species that release their gametes into seawater and that have planktonic larvae capable of dispersing over long distances[70,71]. It is possible to imagine that in abalone and other GASTROPOD mollusc species, reproductive isolation might evolve in the way described below. First, VERL protein changes as a result of a mutation that occurs in one of the 22 VERL repeats. This change might result in a lower affinity of the mutant repeat for lysin, but the mutant repeat is tolerated and fertilization occurs because there are still 21 unchanged VERL repeats in each VERL molecule. So, the redundant nature of VERL leads to relaxed selection on each repeat unit, such that mutations, whether they be beneficial or harmful, do not have any fitness consequences — such tolerance has been suggested for gamete recognition in sea urchins[34]. In successive generations, concerted evolution randomly

GENE CONVERSION
The non-reciprocal transfer of information between homologous genes as a consequence of heteroduplex formation followed by repair mismatches.

GASTROPOD
A class in the phylum Mollusca that is characterized by a muscular foot, on which the body rests, and a single shell. Examples include snails, limpets, sea hares and abalone.

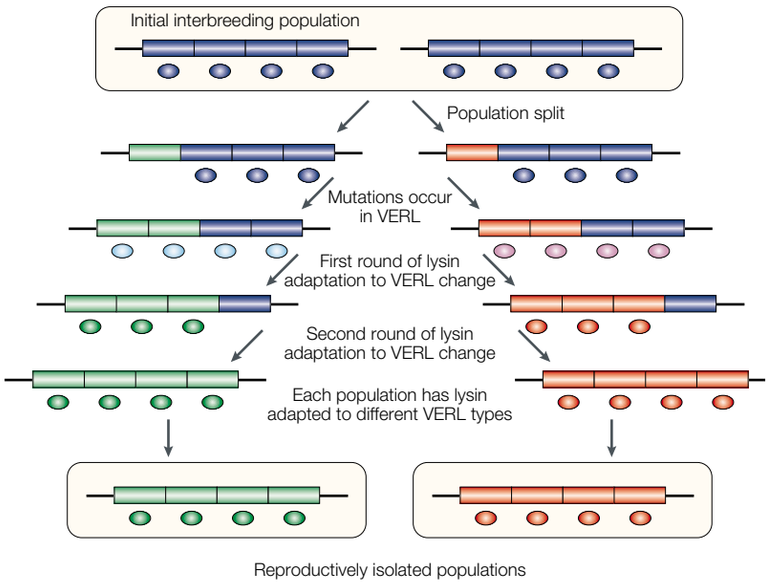


Figure 2 | **Lysin–VERL coevolution might lead to the evolution of species-specific fertilization.** VERL is represented as coloured bars, lysin as coloured circles. A population starts off with one VERL and one lysin type. By chance, mutations in VERL might occur in different populations. With only one changed VERL repeat, lysin might not have to change because it can still interact with the other 21 repeat units. However, unequal crossing over and gene conversion might homogenize the VERL repeat array with the new type. As the new VERL types become more prevalent, lysin will adapt to this change to maintain an efficient VERL–lysin interaction. At the initial stages, when both the new and the old repeat variants are present in the repeat array at equal frequency, lysin might have to adapt to interact with both types. As the new VERL type becomes dominant in the array, lysin could adapt just to that dominant repeat type. So, multiple rounds of adaptation in lysin might correspond to one change in egg VERL.

spreads the mutant repeat within the VERL gene by unequal crossing over and gene conversion[30,66,67]. This creates a continuous selective pressure on lysin to adapt to the ever-changing VERL (FIG. 2) and provides an explanation for the adaptive evolution of lysin[25,26]. This is the only hypothesis based on sequence data that explains the co-evolution of pairs of proteins that are involved in gamete recognition. It explains the maintenance of species-specific fertilization throughout the evolution of species. If a change of habitat, its preference or climate change[72] splits one species of abalone into two populations, the co-evolution of VERL and lysin sequences could follow independent paths in the two populations, which leads to changes in gamete recognition. So, reproductive isolation and subsequent speciation might arise in the abalone as a by-product of the continuous adaptation of lysin to an ever-changing VERL.

The continuous co-evolution of lysin and VERL could also occur within a population. At the extremes of the species' geographical range, or in slightly different habitats, one population might split into two, each becoming reproductively isolated. Given sufficient time, incompatibility at the level of the gamete surface interactions and consequent reproductive isolation would be followed by differentiation of the genomes of the two new species by genetic drift. Natural selection will favour sperm that carry mutations bringing about stronger interactions with new forms of VERL. A favourable mutant lysin might rapidly sweep through the population and become fixed, as indicated by the lack of POLYMORPHISM of lysin genes in individuals of the red abalone species (*Haliotis rufescens*)[25].

Evidence for the above hypothesis comes both from experimental data and theoretical modelling. When the last VERL repeat in the array of 22 repeats was identified and sequenced from 11 pink abalone (*Haliotis corrugata*) individuals from the same location, two variants of VERL repeat sequences were found. Five individuals were homozygous for each of the two variants and only one was heterozygous for both variants[31]. The small number of heterozygotes indicates that ASSORTATIVE MATING might take place in this population of pink abalone. In theory, this molecular differentiation could eventually lead to a sympatric speciation event — the splitting of the current pink abalone population into two new species. However, larger samples of abalone need to be analysed before any firm conclusions can be drawn. It is worth noting that theoretical models have shown that sympatric speciation can occur as a result of SEXUAL SELECTION[73,74].

A similar assortative mating phenomenon has also been found in the *Echinometra* sea urchins. Individual *E. mathaei* have two alleles of bindin, and homozygotes for each variant can be distinguished by PCR and restriction mapping. The eggs of *E. mathaei* are fertilized preferentially by sperm that carry the same bindin allele[75]. This result indicates that the genes that encode bindin and its egg-surface receptor might be linked and inherited as one unit, as is the case in reproductive gene pairs in fungi[17] and plants[18,19]. Quantitative fertilization specificity has also been documented in the sea urchin *Strongylocentrotus pallidus*[76] and other *Echinometra* species[77], which indicates that the differentiation of the gamete-recognition system might have a crucial role in reproductive isolation in many sea-urchin genera.

Theoretical studies that involve computer simulations, which are based on at least one quantitative difference between individuals, support the possibility of speciation in the absence of physical barriers[73,74,78–80]. In one model, sympatric speciation occurs as an outcome of competition for resources[78]. A second model shows that assortative mating can arise in the absence of natural selection[79]. And a third model shows that sympatric speciation can occur when two traits, such as colour and size, are allowed to co-vary[80]. Finally, theoretical models have shown that sympatric speciation can be caused by sexual selection for variation in a male secondary sexual characteristic, such as male coloration, even in a uniform environment[74]. Although these models have not been explicitly developed for reproductive proteins, these proteins could be considered as quantitative traits. Furthermore, other models that are specifically based on reproductive proteins confirm that rapid evolution could result in speciation[81,82].

What drives reproductive protein evolution?

Although distinct evolutionary forces might act in different organisms, the rapid evolution of reproductive proteins seems to occur in several diverse taxonomic groups (TABLE 1). We propose that the selective forces of

POLYMORPHISM
Occurrence, at a single genetic locus, of two or more alleles that differ in nucleotide sequence.

ASSORTATIVE MATING
Non-random mating; it occurs when individuals select their mates on the basis of one or more physical or chemical characteristics.

SEXUAL SELECTION
Selection for characteristics that enhance mating success.

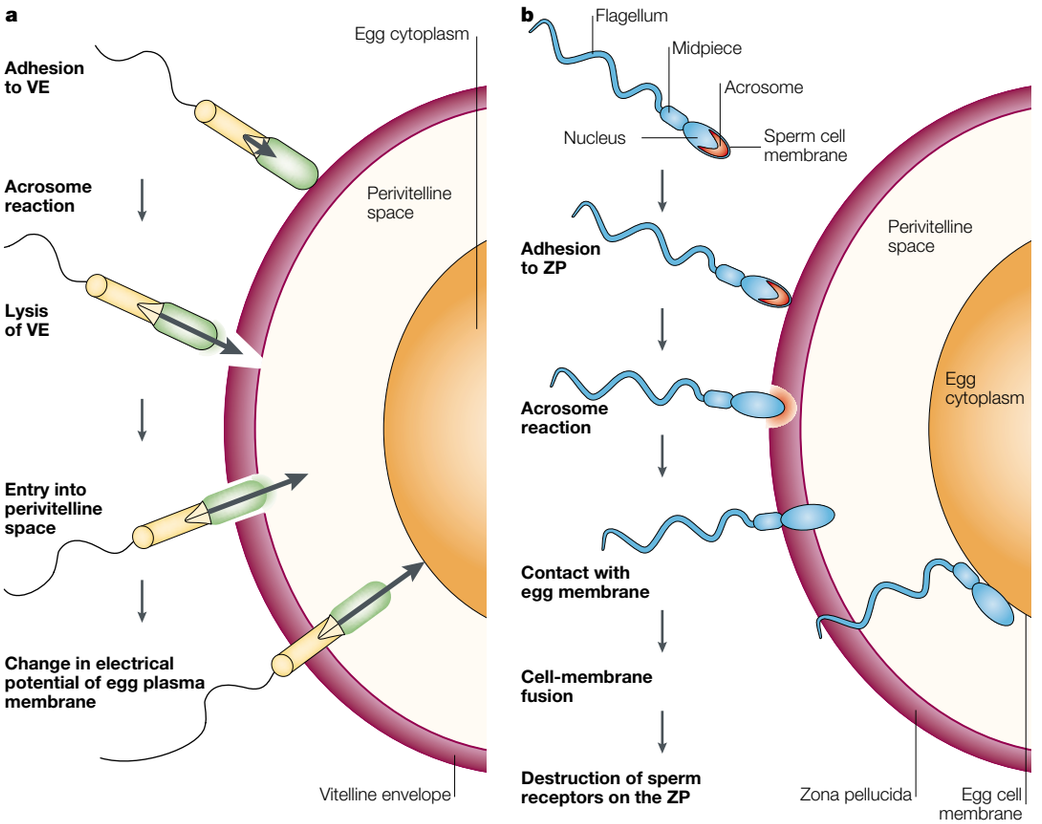


Figure 3 | **The main events in the sperm–egg interaction. a** | In an invertebrate, such as the abalone, the egg is contained within a tough, protective, elevated envelope, called the vitelline envelope (VE). First, the sperm plasma membrane that covers the sperm acrosomal vesicle (AV) adheres to the VE. The sperm AV opens (termed the 'acrosome reaction') and lysin is released onto the VE. Lysin binds to VERL molecules that comprise the VE. The VERL molecules lose cohesion to each other and unravel, which creates a hole in the VE for sperm passage. At the same time, the acrosomal process (AP) lengthens by actin polymerization and becomes coated with the fusagenic AV protein, sp18. The tip of the AP fuses with the egg plasma membrane and the contractile protein network of the egg pulls the sperm into its cytoplasm. The electrical potential of the egg plasma membrane changes to prevent other sperm from fusing with the egg. **b** | In mammals, the egg is contained within an elevated, protective envelope called the zona pellucida (ZP), composed of three glycoproteins — ZP1, ZP2 and ZP3. The sperm membrane binds to ZP3, an event that induces the acrosome reaction. This causes the sperm to bind tightly to ZP2, and enzymes from the AV digest a slit in the ZP through which the sperm swims to reach the egg surface. The membrane that covers the posterior part of the sperm head, known as the 'equatorial segment', then fuses with the egg plasma membrane. The cytoskeletal apparatus of the egg then draws the sperm into its cytoplasm. There is no large change in the electric potential of the egg membrane.

sperm competition, sexual selection and sexual conflict, could individually, or in combination, provide the selective force that drives the rapid evolution of reproductive proteins. Sperm competition[83] (also referred to as sperm precedence[84]) occurs because each sperm competes with all the other sperm to be the first to fuse with the egg. This competition can be fierce; for example, in the male sea urchin there are 200 billion sperm cells per 5 ml of semen. Sperm competition can exert a selective pressure at many steps in the fertilization cascade. Individual sperm could be selected for being the best or the fastest to initiate and maintain swimming, responding to chemoattractants that diffuse from the egg, binding to the egg's surface, binding to the egg components that induce the acrosome reaction, penetrating the egg envelope or fusing with the egg (FIG. 3).

Sexual selection at this cellular level is known as cryptic female choice[85], and it might come into play when an egg prefers to bind to a sperm that carries a particular allele of a sperm-surface protein, whereas another egg has little affinity for that same sperm type. The preference of *Echinometra* eggs to be fertilized by sperm that carry the same bindin allele as they do is a good example[75].

Sexual conflict could come into play when sperm cells are too abundant[86]. Sperm competition presents some problems for the egg, because, for example, it must prevent fusion with more than one sperm (polyspermy). If polyspermy occurs, development and the egg's potential to form an embryo will not be realized. In many animal species, such as frogs, sea urchins, worms and abalone, the first sperm to fuse with the egg sets off a rapid reversal of the electrical potential of the egg membrane, which prevents fusion with other sperm[87]. The electrical block to polyspermy is an excellent example of a QUANTITATIVE TRAIT that might have been selected for by sexual conflict. So, in eggs that are capable of setting up electrical blocks

QUANTITATIVE TRAIT
A measurable trait that depends on the cumulative action of many genes (or quantitative trait loci).

to polyspermy, such as the eggs of abalone[88], it might not be expected that adaptive evolution works on the genes of the egg envelope. As expected, VERL of abalone evolves neutrally[30,31]. In contrast to invertebrate eggs, mammalian eggs do not use an electrical block against polyspermy[87,89]. Therefore, it might be expected that, in mammals, the adaptive evolution of egg coat proteins (ZPs) might regulate sperm receptivity to prevent polyspermy. Surprisingly, the mammalian egg coat proteins ZP3 and ZP2 do show adaptive evolution[10]. It might be that the adaptive evolution of the mammalian ZP2 and ZP3 is driven by the need to adapt to their ever-changing sperm-protein partners. The important point is that one member of the pair of sperm- and egg-surface proteins changes first, and the other member adapts to the change to maximize their interaction.

The above empirical data show the generality of the phenomenon of rapidly evolving reproductive proteins. But what is the theoretical outcome of the continual coevolution of pairs of gamete-recognition proteins? Computer models show that sexual conflict can rapidly lead to speciation by driving the continual evolution of traits that are responsible for reproductive isolation, such as gamete-recognition proteins[81]. Sexual conflict results in the evolution of female reproductive traits to reduce the cost of mating, which might lead to the coevolution of exaggerated male reproductive traits, such as elaborate male coloration[90]. So, both empirical and theoretical studies indicate that the rapid evolution of reproductive proteins could be a driving force in speciation[91–93].

Future directions

A decade ago, we would never have imagined that the sequences of reproductive proteins from closely related species would be so divergent and that their evolution would be directed by an adaptive change. Pairs of gamete-recognition proteins represent examples of mate recognition systems at the cellular level, and rapid evolution seems to be a hallmark of such systems. This rapid evolution occurs in unicellular organisms, such as diatoms with little or no pre-mating barriers[15], and also in mammals with complex mating behaviours[10,54]. In a few cases, such as *Drosophila* accessory-gland proteins, rapid evolution seems to be related to functional differences that are associated with reproductive success[94].

There is more interest today in the molecular biology of reproduction than at any time in the past. Although we feel that the foundation of the basic phenomena that many reproductive proteins evolve rapidly has been laid, much more work needs to be done. More comparative sequence information from vertebrate pairs of sperm–egg proteins is needed, as are comparisons of gamete-recognition proteins from species with different mating strategies. It would also be interesting to compare the rates of evolution of reproductive proteins between species with multiple matings and those with single matings. The sexual conflict hypothesis would predict that evolution would be more rapid in the species with multiple matings because an increased mating rate escalates the conflict[95]. Sequences of reproductive proteins from a wider variety of species must be surveyed. We must look for sequence differences in reproductive proteins within the same population of the same species, and compare sympatric and ALLOPATRIC species. Analyses of reproductive proteins that are not rapidly evolving might also provide clues into why some other reproductive genes do evolve rapidly[96]. Most importantly, functional studies are needed to determine the consequences of the rapid evolution of reproductive proteins. Genomics, proteomics and advances in sequencing methods, as well as sequence analysis, will allow the accumulation of much more data; these will help to clarify why reproductive proteins show such extensive sequence divergence, and the role of this divergence in the speciation process.

ALLOPATRIC
Having non-overlapping geographical distributions.

1. Singh, R. S. & Kulathinal, R. J. Sex gene pool evolution and speciation: a new paradigm. *Genes Genet. Syst.* **75**, 119–130 (2000).
2. Vacquier, V. D. Evolution of gamete recognition proteins. *Science* **281**, 1995–1998 (1998).
3. Civetta, A. & Singh, R. S. High divergence of reproductive tract proteins and their association with postzygotic reproductive isolation in *Drosophila melanogaster* and *Drosophila virilis* group species. *J. Mol. Evol.* **41**, 1085–1095 (1995).
An important paper showing that, on average, *Drosophila* reproductive proteins are about twice as diverse as non-reproductive proteins.
4. Yang, Z. & Bielawski, J. P. Statistical methods for detecting molecular adaptation. *Trends Ecol. Evol.* **15**, 496–503 (2000).
An excellent review on the use of the d_N/d_S ratio to detect adaptive evolution (positive Darwinian selection).
5. Makalowski, W. & Boguski, M. S. Evolutionary parameters of the transcribed mammalian genome: an analysis of 2,820 orthologous rodent and human sequences. *Proc. Natl Acad. Sci. USA* **95**, 9407–9412 (1998).
The authors compare many genes between rodents and humans, and provide a good database for the comparison of rapidly, and non-rapidly, evolving genes.
6. Li, W.-H. *Molecular Evolution* (Sinauer Associates, Sunderland, Massachusetts, 1997).
7. Lee, Y. H., Ota, T. & Vacquier, V. D. Positive selection is a general phenomenon in the evolution of abalone sperm lysin. *Mol. Biol. Evol.* **12**, 231–238 (1995).
8. Hughes, A. L. & Nei, M. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature* **335**, 167–170 (1988).
9. Yang, Z., Nielsen, R., Goldman, N. & Pedersen, A. M. Codon-substitution models for heterogeneous selection pressure at amino acid sites. *Genetics* **155**, 431–449 (2000).
10. Swanson, W. J., Yang, Z., Wolfner, M. F. & Aquadro, C. F. Positive Darwinian selection drives the evolution of several female reproductive proteins in mammals. *Proc. Natl Acad. Sci. USA* **98**, 2509–2514 (2001).
This is the first demonstration of female reproductive proteins being subject to positive Darwinian selection.
11. Swanson, W. J., Clark, A. G., Waldrip-Dail, H. M., Wolfner, M. F. & Aquadro, C. F. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 7375–7379 (2001).
12. Luporini, P., Vallesi, A., Miceli, C. & Bradshaw, R. A. Chemical signaling in ciliates. *J. Eukaryot. Microbiol.* **42**, 208–212 (1995).
13. Ferris, P. J., Pavlovic, C., Fabry, S. & Goodenough, U. W. Rapid evolution of sex-related genes in *Chlamydomonas*. *Proc. Natl Acad. Sci. USA* **94**, 8634–8639 (1997).
14. Ferris, P. J., Woessner, J. P. & Goodenough, U. W. A sex recognition glycoprotein is encoded by the plus mating-type gene *fus1* of *Chlamydomonas reinhardtii*. *Mol. Biol. Cell* **7**, 1235–1248 (1996).
15. Armbrust, E. V. & Galindo, H. M. Rapid evolution of a sexual reproduction gene in centric diatoms of the genus *Thalassiosira*. *Appl. Environ. Microbiol.* **67**, 3501–3513 (2001).
16. Brown, A. J. & Casselton, L. A. Mating in mushrooms: increasing the chances but prolonging the affair. *Trends Genet.* **17**, 393–400 (2001).
17. Casselton, L. A. & Olesnicky, N. S. Molecular genetics of mating recognition in basidiomycete fungi. *Microbiol. Mol. Biol. Rev.* **62**, 55–70 (1998).
18. Schopfer, C. R., Nasrallah, M. E. & Nasrallah, J. B. The male determinant of self-incompatibility in *Brassica*. *Science* **286**, 1697–1700 (1999).
19. Schopfer, C. R. & Nasrallah, J. B. Self-incompatibility. Prospects for a novel putative peptide-signaling molecule. *Plant Physiol.* **124**, 935–940 (2000).
20. Kachroo, A., Schopfer, C. R., Nasrallah, M. E. & Nasrallah, J. B. Allele-specific receptor–ligand interactions in *Brassica* self-incompatibility. *Science* **293**, 1824–1826 (2001).
An elegant biochemical demonstration of receptor–ligand interaction that shows the specificity of reproductive proteins.
21. Nasrallah, J. B. Cell–cell signaling in the self-incompatibility response. *Curr. Opin. Plant Biol.* **3**, 368–373 (2000).
22. Richman, A. D. & Kohn, J. R. Evolutionary genetics of self-incompatibility in the Solanaceae. *Plant Mol. Biol.* **42**, 169–179 (2000).
23. Mayfield, J. A., Fiebig, A., Johnstone, S. E. & Preuss, D. Gene families from the *Arabidopsis thaliana* pollen coat proteome. *Science* **292**, 2482–2485 (2001).
24. Hellberg, M. E. & Vacquier, V. D. Rapid evolution of fertilization selectivity and lysin cDNA sequences in teguline gastropods. *Mol. Biol. Evol.* **16**, 839–848 (1999).
25. Metz, E. C., Robles-Sikisaka, R. & Vacquier, V. D. Nonsynonymous substitution in abalone sperm fertilization genes exceeds substitution in introns and mitochondrial DNA. *Proc. Natl Acad. Sci. USA* **95**, 10676–10681 (1998).

26. Yang, Z., Swanson, W. J. & Vacquier, V. D. Maximum-likelihood analysis of molecular adaptation in abalone sperm lysin reveals variable selective pressures among lineages and sites. *Mol. Biol. Evol.* **17**, 1446–1455 (2000).
27. Swanson, W. J. & Vacquier, V. D. Liposome fusion induced by a M(r) 18,000 protein localized to the acrosomal region of acrosome-reacted abalone spermatozoa. *Biochemistry* **34**, 14202–14208 (1995).
28. Swanson, W. J. & Vacquier, V. D. Extraordinary divergence and positive Darwinian selection in a fusagenic protein coating the acrosomal process of abalone spermatozoa. *Proc. Natl Acad. Sci. USA* **92**, 4957–4961 (1995).
29. Hellberg, M. E., Moy, G. W. & Vacquier, V. D. Positive selection and propeptide repeats promote rapid interspecific divergence of a gastropod sperm protein. *Mol. Biol. Evol.* **17**, 458–466 (2000).
30. Swanson, W. J. & Vacquier, V. D. Concerted evolution in an egg receptor for a rapidly evolving abalone sperm protein. *Science* **281**, 710–712 (1998).
A model of species-specific fertilization based on molecular evolutionary analysis of interacting male and female reproductive proteins in plants.
31. Swanson, W. J., Aquadro, C. F. & Vacquier, V. D. Polymorphism in abalone fertilization proteins is consistent with the neutral evolution of the egg's receptor for lysin (VERL) and positive Darwinian selection of sperm lysin. *Mol. Biol. Evol.* **18**, 376–383 (2001).
32. Vacquier, V. D., Swanson, W. J. & Hellberg, M. E. What have we learned about sea urchin sperm bindin. *Dev. Growth Differ.* **37**, 1–10 (1995).
33. Palumbi, S. R. & Metz, E. C. Strong reproductive isolation between closely related tropical sea urchins (genus *Echinometra*). *Mol. Biol. Evol.* **8**, 227–239 (1991).
34. Metz, E. C. & Palumbi, S. R. Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. *Mol. Biol. Evol.* **13**, 397–406 (1996).
35. Biermann, C. H. The molecular evolution of sperm bindin in six species of sea urchins (*Echinodia*: *Strongylocentrotidae*). *Mol. Biol. Evol.* **15**, 1761–1771 (1998).
36. Wolfner, M. F. Tokens of love: functions and regulation of *Drosophila* male accessory gland products. *Insect Biochem. Mol. Biol.* **27**, 179–192 (1997).
A review of *Drosophila* accessory-gland proteins, which have been included in several evolutionary studies of reproductive proteins.
37. Partridge, L. & Hurst, L. D. Sex and conflict. *Science* **281**, 2003–2008 (1998).
38. Xue, L. & Noll, M. *Drosophila* female sexual behavior induced by sterile males showing copulation complementation. *Proc. Natl Acad. Sci. USA* **97**, 3272–3275 (2000).
39. Chen, P. S. *et al.* A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell* **54**, 291–298 (1988).
40. Herndon, L. A. & Wolfner, M. F. A *Drosophila* seminal fluid protein, Acp26Aa, stimulates egg laying in females for 1 day after mating. *Proc. Natl Acad. Sci. USA* **92**, 10114–10118 (1995).
41. Heifetz, Y., Lung, O., Frongillo, E. A. Jr & Wolfner, M. F. The *Drosophila* seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. *Curr. Biol.* **10**, 99–102 (2000).
42. Kalb, J. M., DiBenedetto, A. J. & Wolfner, M. F. Probing the function of *Drosophila melanogaster* accessory glands by directed cell ablation. *Proc. Natl Acad. Sci. USA* **90**, 8093–8097 (1993).
43. Bertram, M. J., Neubaum, D. M. & Wolfner, M. F. Localization of the *Drosophila* male accessory gland protein Acp36DE in the mated female suggests a role in sperm storage. *Insect Biochem. Mol. Biol.* **26**, 971–980 (1996).
44. Neubaum, D. M. & Wolfner, M. F. Mated *Drosophila melanogaster* females require a seminal fluid protein, Acp36DE, to store sperm efficiently. *Genetics* **153**, 845–857 (1999).
45. Tram, U. & Wolfner, M. F. Male seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. *Genetics* **153**, 837–844 (1999).
46. Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**, 241–244 (1995).
47. Harshman, L. G. & Prout, T. Sperm displacement without sperm transfer in *Drosophila melanogaster*. *Evolution* **48**, 758–766 (1994).
48. Clark, A. G., Aguade, M., Prout, T., Harshman, L. G. & Langley, C. H. Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. *Genetics* **139**, 189–201 (1995).
49. Tsaur, S. C. & Wu, C. I. Positive selection and the molecular evolution of a gene of male reproduction, Acp26Aa of *Drosophila*. *Mol. Biol. Evol.* **14**, 544–549 (1997).
50. Tsaur, S. C., Ting, C. T. & Wu, C. I. Positive selection driving the evolution of a gene of male reproduction, *Acp26Aa*, of *Drosophila*. II. Divergence versus polymorphism. *Mol. Biol. Evol.* **15**, 1040–1046 (1998).
51. Begun, D. J., Whitley, P., Todd, B. L., Waldrip-Dail, H. M. & Clark, A. G. Molecular population genetics of male accessory gland proteins in *Drosophila*. *Genetics* **156**, 1879–1888 (2000).
52. Aguade, M. Positive selection drives the evolution of the Acp29AB accessory gland protein in *Drosophila*. *Genetics* **152**, 543–551 (1999).
53. Fuyama, Y. Species-specificity of paragonial substances as an isolating mechanism in *Drosophila*. *Experientia* **39**, 190–192 (1983).
54. Wyckoff, G. J., Wang, W. & Wu, C. I. Rapid evolution of male reproductive genes in the descent of man. *Nature* **403**, 304–309 (2000).
This paper shows that rapidly evolving reproductive proteins occur in organisms with complex mating courtships.
55. Wassarman, P. M. Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion. *Cell* **96**, 175–183 (1999).
56. Yurewicz, E. C., Sacco, A. G., Gupta, S. K., Xu, N. & Gage, D. A. Hetero-oligomerization-dependent binding of pig oocyte zona pellucida glycoproteins ZPB and ZPC to boar sperm membrane vesicles. *J. Biol. Chem.* **273**, 7488–7494 (1998).
57. Chen, J., Litscher, E. S. & Wassarman, P. M. Inactivation of the mouse sperm receptor, mZP3, by site-directed mutagenesis of individual serine residues located at the combining site for sperm. *Proc. Natl Acad. Sci. USA* **95**, 6193–6197 (1998).
58. Eidne, K. A., Henery, C. C. & Aitken, R. J. Selection of peptides targeting the human sperm surface using random peptide phage display identify ligands homologous to ZP3. *Biol. Reprod.* **63**, 1396–1402 (2000).
59. Gao, Z. & Garbers, D. L. Species diversity in the structure of zonadhesin, a sperm-specific membrane protein containing multiple cell adhesion molecule-like domains. *J. Biol. Chem.* **273**, 3415–3421 (1998).
60. Juneja, R., Agulnik, S. I. & Silver, L. M. Sequence divergence within the sperm-specific polypeptide TCTE1 is correlated with species-specific differences in sperm binding to zona-intact eggs. *J. Androl.* **19**, 183–188 (1998).
61. Higgie, M., Chenoweth, S. & Blows, M. W. Natural selection and the reinforcement of mate recognition. *Science* **290**, 519–521 (2000).
62. Hill, K. L. & L'Hernault, S. W. Analyses of reproductive interactions that occur after heterospecific matings within the genus *Caenorhabditis*. *Dev. Biol.* **232**, 105–114 (2001).
63. O'Rand, M. G. Sperm–egg recognition and barriers to interspecies fertilization. *Gamete Res.* **19**, 315–328 (1988).
64. Swanson, W. J. & Vacquier, V. D. The abalone egg vitelline envelope receptor for sperm lysin is a giant multivalent molecule. *Proc. Natl Acad. Sci. USA* **94**, 6724–6729 (1997).
65. Kresge, N., Vacquier, V. D. & Stout, C. D. Abalone lysin: the dissolving and evolving sperm protein. *Bioessays* **23**, 95–103 (2001).
66. Elder, J. F. Jr & Turner, B. J. Concerted evolution of repetitive DNA sequences in eukaryotes. *Q. Rev. Biol.* **70**, 297–320 (1995).
67. McAllister, B. F. & Werren, J. H. Evolution of tandemly repeated sequences: what happens at the end of an array? *J. Mol. Evol.* **48**, 469–481 (1999).
68. Wu, C. I. The genic view of the process of speciation. *J. Evol. Biol.* **14**, 851–865 (2001).
69. Rieseberg, L. H., Van, F. C. & Desrochers, A. M. Hybrid speciation accompanied by genomic reorganization in wild sunflowers. *Nature* **375**, 313–316 (1995).
70. Knowlton, N. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* **420**, 73–90 (2000).
71. Palumbi, S. R. Marine speciation on a small planet. *Trends Ecol. Evol.* **7**, 114–118 (1992).
72. Hellberg, M. E., Balch, D. P. & Roy, K. Climate-driven range expansion and morphological evolution in a marine gastropod. *Science* **292**, 1707–1710 (2001).
73. Van Doorn, G. S. Ecological versus sexual selection models of sympatric speciation. *Selection* (in the press).
74. Higashi, M., Takimoto, G. & Yamamura, N. Sympatric speciation by sexual selection. *Nature* **402**, 523–526 (1999).
75. Palumbi, S. R. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proc. Natl Acad Sci. USA* **96**, 12632–12637 (1999).
This paper shows the functional consequences of diversity of reproductive proteins, and that fertilization efficiency is dependent on the genotype of the reproductive protein loci.
76. Biermann, C. H. & Marks, J. A. Geographic divergence of gamete recognition systems in two species in the sea urchin genus *Strongylocentrotus*. *Zygote* **8**, S86–S87 (2000).
77. Rahman, M. A. & Uehara, T. Experimental hybridisation between two tropical species of sea urchins (genus *Echinometra*) in Okinawa. *Zygote* **8**, S90 (2000).
78. Dieckmann, U. & Doebeli, M. On the origin of species by sympatric speciation. *Nature* **400**, 354–357 (1999).
79. Kondrashov, A. S. & Shpak, M. On the origin of species by means of assortative mating. *Proc. R. Soc. Lond.* B **265**, 2273–2278 (1998).
80. Kondrashov, A. S. & Kondrashov, F. A. Interactions among quantitative traits in the course of sympatric speciation. *Nature* **400**, 351–354 (1999).
81. Gavrilets, S. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**, 886–889 (2000).
A mathematical model that shows how the rapid evolution of genes, such as in those that encode reproductive proteins, can lead to speciation.
82. Wu, C. I. A stochastic simulation study on speciation by sexual selection. *Evolution* **39**, 66–82 (1985).
83. Clark, A. G., Begun, D. J. & Prout, T. Female × male interactions in *Drosophila* sperm competition. *Science* **283**, 217–220 (1999).
84. Howard, D. J. Conspecific sperm and pollen precedence and speciation. *A. Rev. Ecol. Syst.* **30**, 109–132 (1999).
85. Eberhard, W. G. *Female Control: Sexual Selection by Cryptic Female Choice* (Princeton Univ. Press, New Jersey, 1996).
86. 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).
87. Gould-Somero, M. & Jaffe, L. A. in *Cell Fusion and Transformation* (eds Beers, R. F. & Bassett, E. G.) 27–38 (Raven, New York, 1984).
88. Stephano, J. L. in *Abalones of the World* (eds Shepherd, S. A., Tegner, M. J. & Guzman del Proo, S. A.) 518–526 (Blackwell Science, Cambridge, Massachussetts,1992).
89. Jaffe, L. A., Sharp, A. P. & Wolf, D. P. Absence of an electrical polyspermy block in the mouse. *Dev. Biol.* **96**, 317–323 (1983).
90. Gavrilets, S., Arnqvist, G. & Friberg, U. The evolution of female mate choice by sexual conflict. *Proc. R. Soc. Lond.* B **268**, 531–539 (2001).
91. Barton, N. The rapid origin of reproductive isolation. *Science* **290**, 462–463 (2000).
92. Eady, P. E. Postcopulatory, prezygotic reproductive isolation. *J. Zool.* **253**, 47–52 (2001).
93. Blows, M. W. Evolution of the genetic covariance between male and female components of mate recognition: an experimental test. *Proc. R. Soc. Lond.* B **266**, 2169–2174 (1999).
94. Rice, W. R. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**, 232–234 (1996).
A description of a laboratory experiment showing that sexual conflict might drive the rapid evolution of reproductive proteins.
95. Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. Sexual conflict promotes speciation in insects. *Proc. Natl Acad. Sci. USA* **97**, 10460–10464 (2000).
96. Metz, E. C., Gomez-Gutierrez, G. & Vacquier, V. D. Mitochondrial DNA and bindin gene sequence evolution among allopatric species of the sea urchin genus *Arbacia*. *Mol. Biol. Evol.* **15**, 185–195 (1998).

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This article will appear as part of a web focus on the evolution of sex, which will coincide with our forthcoming special issue on this topic.

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