2. Jeffreys, A.J., Royle, N.J., Wilson, V., and Wong, Z. (1988). Spontaneous mutation-rates to new length alleles at tandem-repetitive hypervariable loci in Human DNA. Nature *332*, 278–281.
3. Ellegren, H. (2000). Heterogeneous mutation processes in human microsatellite DNA sequences. Nat. Genet. *24*, 400–402.
4. Xu, X., Peng, M., Fang, Z., and Xu, X. (2000). The direction of microsatellite mutations is dependent upon allele length. Nat. Genet. *24*, 396–399.
5. Howell, N., Kubacka, I., and Mackey, D.A. (1996). How rapidly does the human mitochondrial genome evolve? Am. J. Hum. Genet. *59*, 501–509.
6. Parsons, T.J., Muniec, D.S., Sullivan, K., Woodyatt, N., Alliston-Greiner, R., Wilson, M.R., Berry, D.L., Holland, K.A., Weedn, V.W., Gill, P., *et al*. (1997). A high observed substitution rate in the human mitochondrial DNA control region. Nat. Genet. *15*, 363–368.
7. Sigurðardóttir, S., Helgason, A., Gulcher, J.R., Stefansson, K., and Donnelly, P. (2000). The mutation rate in the human mtDNA control region. Am. J. Hum. Genet. *66*, 1599–1609.
8. Jeffreys, A.J., Neumann, R., and Wilson, V. (1990). Repeat unit sequence variation in minisatellites - a novel source of DNA polymorphism for studying variation and mutation by single molecule analysis. Cell *60*, 473–485.
9. Jeffreys, A.J., Tamaki, K., Macleod, A., Monckton, D.G., Neil, D.L., and Armour, J.A.L. (1994). Complex gene conversion events in germline mutation at human minisatellites. Nat. Genet. *6*, 136–145.
10. Turner, D.J., Miretti, M., Rajan, D., Fiegler, H., Carter, N.P., Blayney, M.L., Beck, S., and Hurles, M.E. (2008). Germline rates of de novo meiotic deletions and duplications causing several genomic disorders. Nat. Genet. *40*, 90–95.

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# Evolution: No-Male's Land for an Amazonian Ant

**Recent work has shown that, in the Amazonian fungus-growing ant *Mycocepurus smithii*, queens use exclusively asexual reproduction and the male sex seems to have disappeared from the species. This finding illustrates the remarkable diversity of reproductive systems in ants.**

**Denis Fournier and Serge Aron**

Sexual reproduction largely predominates in animals and plants. This is considered a paradox because sexually reproducing females transmit only half of their genome to their progeny and produce only half as many female offspring than asexually reproducing females, which do not have to produce males to ensure reproduction [1]. This two-fold cost of sexual reproduction is counterbalanced by the fact that sex and recombination promote genetic variation among progeny. This is supposed to provide an advantage in coevolutionary arms races, particularly against parasites, and also to facilitate the purging of deleterious mutations [2]. A recent work by Himler *et al.* [3] shows that the fungus-growing ant *Mycocepurus smithii* has evolved a reproductive system where males have disappeared and sex is forsaken.

In a combination of genetic, morphological and antibiotic experiments, Himler *et al.* [3] describe the first case of complete asexuality in ants. Microsatellite DNA fingerprinting based on a diallelic locus (over 14 tested) showed that female offspring — workers and reproductive queens — share an identical genotype with their mother, consistent with clonal reproduction through parthenogenesis (thelytoky). Dissections of the female reproductive tracts confirmed that queens are not inseminated albeit they are active egg layers. The mussel organ, an internal lock structure of the female mating apparatus, was found to be degenerated and unsclerotized, thereby preventing male's genitalia from locking during mating. Asexuality in *M. smithii* is not caused by infection with endosymbiotic bacteria or by vertically transmitted exosymbionts — it was not possible to induce male production by antibiotic treatment or fungal substitution [3].

Consistent with these findings, extensive field surveys throughout Latin America and laboratory rearing during several years have failed to put a hand on a single male [4,5]. Furthermore, behaviours specific to the mating period — modifications of the nest structure, synchrony in production of female sexuals — were never observed in *M. smithii*, whereas they occur in the sympatric sexual species *M. goeldii* [5].

Clonal reproduction was previously shown to occur in other ants belonging to four phylogenetically distant subfamilies (Myrmicinae, Formicinae, Cerapachyinae and Ponerinae). Recent findings on three such species — *Cataglyphis cursor* [6], *Wasmannia auropunctata* [7] and *Vollenhovia emeryi* [8] — showed that queens circumvent the two-fold cost of sex by using alternative modes of reproduction for the production of reproductive and non-reproductive female offspring. In these species, new queens are produced almost exclusively by thelytokous parthenogenesis, whereas workers are produced by sexual reproduction. Furthermore, in the rare ant species where the queen caste is absent or morphologically reduced, the workers obligatorily produce diploid females by parthenogenesis [9,10]; there are three species known where the queen caste is usually present, suppressing worker reproduction, but when the queen dies or is removed from the nest for some reason, the workers can then lay female eggs through thelytokous parthenogenesis [11–13]. Although males occur and take part in reproduction in all of these species, *M. smithii* would seem to be the only one where males are totally absent.

Sexual reproduction may lead to conflicts between sexes when characteristics that enhance the reproductive success of one sex reduce the fitness of the opposite sex [14]. The use of both sexual and asexual reproduction to produce workers and reproductive females, respectively, maximizes the reproductive success of queens by increasing the transmission rate of their genes to their reproductive daughters, while maintaining genetic diversity in the worker force. But this mode of reproduction considerably decreases the fitness of males who never father queens. In ants, as in other

Hymenoptera, females are usually produced by sexual reproduction and are diploid, whereas males develop from unfertilised eggs and are haploid. Thus, males never father sons and they achieve their reproductive success only through reproductive female offspring. Until the recent discovery of the reproductive system of *M. smithii*, males appeared to resist, even to thwart, the clonality of queens. In *C. cursor*, males contribute a small proportion of the reproductive queens, as almost 4% of them arise from classical sexual reproduction. In addition, they can indirectly transmit their genome to the queens produced by the thelytokous-laying workers when colonies are naturally orphaned [6]. The situation is more complex in *W. auropunctata*, where all queens of a population are produced clonally and where workers are completely sterile. But in this species, and probably also in *V. emeryi*, males use queens as surrogate mothers to clone themselves. Males could thwart queens by eliminating the female genome in the zygote during brood development, or by fertilizing enucleated ovules accidentally produced by queens [7,15]. In *M. smithii*, males have disappeared. Should one come out of somewhere, it would not be able to mate given that, as mentioned above, the queens have a degenerated mussel organ.

Another biological curiosity of *M. smithii* lies in the fact that, in contrast to most other fungus-growing ants, the fungus they cultivate also reproduces asexually. In both the fungus and its host, it seems that the benefits of clonality outweigh its cost due to reduced genetic diversity and potential resistance to environmental pressures. *M. smithii* could bear genotypes that allow a large degree of phenotypic plasticity and an adequate and sustained level of heterozygosity, a 'general purpose genotype' [3,16]. Interestingly, the ant *W. auropunctata*, one of the most successful invasive species [17], achieves the same goal — adaptation to a given environment — but through a different path. Most clonal couples of *W. auropunctata* are formed by particular combinations of queen and male genotypes originating from genetically differentiated lineages. This results in the production of highly heterozygous workers. High genetic variability among the worker caste might, in turn, enhance their ability to tolerate and exploit a large range of habitat and resources [7,15].

Other biological traits may have favoured the dual asexual symbiosis between the ant and its fungus. Workers of this species grow different varieties of fungi within the same area, rather than just one as other fungus-growing ants do, and switch to distant fungal crops. This generates novel combinations of ant farmer and crop genomes. Complex architecture of nests, consisting of a network of tunnels and chambers protected in the soil, provides a stable microenvironment that allows the maintenance of clonal lineages. Moreover, *M. smithii* may adopt collective behaviours such as grooming, waste management or the use of antibiotic secretions or plant compounds that help in maintaining an auspicious environment [18].

At first sight, the ubiquity of clonal reproduction in *M. smithii* suggests that sex has been forsaken a long time ago in this species [3]. Nevertheless, earlier disappearance of sexual reproduction does not explain why genetic diversity is so low across all study sites sampled, and why organs rendered obsolescent, such as the spermatheca that serve to stock the sperm until fertilization, still occur in the reproductive tract of queens. For these reasons, Rabeling *et al.* [5] argue in favour of a recent origin of thelytoky, followed by a rapid expansion of asexual reproduction in *M. smithii.* Either way, these findings confirm that clonal systems may be associated with a dynamic and adaptive genome. And this raises new interesting questions. What factors might have propelled sexual ancestors towards a life without sex? How does such a clonal system respond to environmental pressures (parasites, diseases)? What are the proxies involved in caste determination — the development of a fertilised egg into either a reproductive queen or a worker?

On a cytological basis, thelytokous parthenogenesis can be divided into two main types: apomictic (or ameiotic) parthenogenesis, where no recombination of alleles occurs and the offspring are 'true clones' of their mother, and automictic (or meiotic) parthenogenesis with possible gene recombination during meiosis. Given that a single diallelic locus is insufficient to detect a signature of a meiotic or an ameiotic parthenogenesis [19], does the genotype of an individual predict caste fate or do environmental effects solely govern caste determination? Or even, do queens still produce haploid, male eggs? Clearly, ants offer evolutionary perspectives of researches that are inexhaustible. Combining behavioural and ecological studies with the advent of genomics will undoubtedly help decipher unexpected reproductive strategies such as those reported for the Amazonian ant *Mycocepurus smithii*.

**References**

1. Maynard Smith, J. (1978). The Evolution of Sex (Cambridge: Cambridge University Press).
2. West, S.A., Lively, C.M., and Read, A.F. (1999). A pluralist approach to sex and recombination. J. Evol. Biol. *12*, 1003–1012.
3. Himler, A.G., Caldera, E.J., Baer, B.C., Fernández-Marín, H., and Mueller, U.G. (2009). No sex in fungus-farming ants or their crops. Proc. R. Soc. Lond. B. *276*, 2611–2616.
4. Fernández-Marín, H., Zimmerman, J.K., Wcislo, W.T., and Rehner, S.A. (2005). Colony foundation, nest architecture and demography of a basal fungus-growing ant, *Mycocepurus smithii* (Hymenoptera, Formicidae). J. Nat. Hist. *39*, 1735–1743.
5. Rabeling, C., Lino-Neto, J., Cappellari, S.C., Dos-Santos, I.A., Mueller, U.G., and Bacci, M., Jr. (2009). Thelytokous parthenogenesis in the fungus-gardening ant *Mycocepurus smithii* (Hymenoptera: Formicidae). PLoS One, in press.
6. Pearcy, M., Aron, S., Doums, C., and Keller, L. (2004). Conditional use of sex and parthenogenesis for worker and queen production in ants. Science *306*, 1780–1783.
7. Fournier, D., Estoup, A., Orivel, J., Foucaud, J., Jourdan, H., Le Breton, J., and Keller, L. (2005). Clonal reproduction by males and females in the little fire ant. Nature *435*, 1230–1234.
8. Ohkawara, K., Nakayama, M., Satoh, A., Trindl, A., and Heinze, J. (2006). Clonal reproduction and genetic caste differences in a queen-polymorphic ant, *Vollenhovia emeryi*. Biol. Lett. *2*, 359–363.
9. Itow, T., Kobayashi, K., Kubota, M., Ogata, K., Imai, H.T., and Crozier, R.H. (1984). The reproductive cycle of the queenless ant *Pristomyrmex pungens*. Insectes Soc. *31*, 87–102.
10. Tsuji, K., and Yamauchi, K. (1995). Production of females by parthenogenesis in the ant *Cerapachys biroi*. Insectes Soc. *42*, 333–336.
11. Cagniant, H. (1973). Apparition d'ouvrières à partir d'oeufs pondus par des ouvrières chez la fourmi *Cataglyphis cursor* Fonscolombe (Hyménoptères, Formicidae). C.R. Acad. Sci. III, Sci. Vie. *277*, 2197–2198.
12. Heinze, J., and Hölldobler, B. (1995). Thelytokous parthenogenesis and dominance hierarchies in the ponerine ant, *Platythyrea punctata*. Naturwissenschaften *82*, 40–41.
13. Grasso, D.A., Wenseleers, T., Mori, A., Le Moli, F., and Billen, J. (2000). Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant

*Messor Capitatus*. Ethol. Ecol. Evol. *12*, 309–314.
14. Arnqvist, G., and Rowe, L. (2005). Sexual Conflict (Princeton, NJ: Princeton University Press).
15. Foucaud, J., Fournier, D., Orivel, J., Delabie, J.H.C., Loiseau, A., Le Breton, J., Kergoat, G.J., and Estoup, A. (2007). Sex and clonality in the little fire ant. Mol. Biol. Evol. *24*, 2465–2473.
16. Baker, H.G. (1965). Characteristics and modes of origin of weeds. In The Genetics of Colonizing Species, H.G. Baker and G.L. Stebbins, eds. (New York: Academic Press), pp. 147–168.
17. Lowe, S., Browne, M., and Boudjelas, S., (2001). 100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database. (Auckland: IUCN-ISSG), p. 11.
18. Christe, P., Oppliger, A., Bancalà, F., Castella, G., and Chapuisat, M. (2003). Evidence for collective medication in ants. Ecol. Lett. *6*, 19–22.
19. Crozier, R.H. (1975). Animal Cytogenetics - 3 Insecta (7) Hymenoptera (Berlin: Gebruder Borntraeger).

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# Protein Evolution: Innovative Chaps

**Mutations in proteins allow functional innovation, but can be critically destabilizing. Recent work shows how chaperonins can rescue innovative mutants, with implications for protein engineering and adaptive evolution.**

**D. Allan Drummond**

Like hikers on a cliff edge, proteins tend to be one step away from a disastrous fall. Over evolutionary time, proteins hike in sequence space [1], with each step corresponding to a sequence change, most often a single amino-acid substitution. Each step offers the opportunity, however unlikely, to acquire improved or novel activity, yet also carries the risk, often quite likely, of becoming unstable (Figure 1A), leading to misfolding and degradation. For protein engineers looking to explore new functions, and perhaps for organisms faced with a new environmental challenge, the most useful substitutions may at the same time be the most disruptive to protein stability [2,3]. Innovation, in short, is costly. Recent work [4] suggests that this cost of innovation can be mitigated by helper proteins called chaperonins which help some of their protein clients to accumulate more, and sometimes more useful, substitutions than they could in the absence of folding assistance.

## Stability to Folding to Activity

Most proteins fold with a net stability equivalent to that of a handful of hydrogen bonds or, importantly, to the effect of a single destabilizing amino acid substitution. Critically destabilized proteins cannot maintain the folded state that confers their biological activity, and tend to aggregate, often observed macroscopically as a sharp decrease in solubility. In a rare study where protein folding and function were measured independently for a large number of enzymes bearing random mutations, almost all folded proteins at least retained parental function [2]. Amino acid changes that preserve stable folding represent opportunities for functional innovation, and indeed, in the same study, a more-stable enzyme variant acquired novel functions at a far higher rate than its less-stable counterpart [2]. Conversely, when directed evolution of a fatty-acid hydroxylase toward activity on short-chain alkanes stalled, it was because the innovative mutations had destabilized the enzyme such that few, if any, additional mutations could be tolerated [5]. Stabilizing the enzyme, however, enabled the mutational march toward new substrates to resume [5]. Another way to accelerate acquisition of new protein functions would be to provide a system to buffer the effects of destabilizing mutations [2], and chaperones offer a perfect example of such a system.

The bacterial chaperonins GroEL and GroES form a cavity in which amino-acid chains can attempt to fold while protected from the crowded intracellular milieu. About 10% of soluble *Escherichia coli* proteins are clients of GroEL/S [6], which are known to suppress a wide range of mutations [7]. By performing mutation-accumulation experiments on four soluble enzymes under conditions where GroEL/S were alternately overexpressed or expressed normally, Tokuriki and Tawfik [4] demonstrated that a larger fraction of mutant proteins retained activity in the presence of high chaperonin levels than at normal levels of expression. Many of the chaperonin-compensated proteins had folding defects appearing as decreased solubility in the absence of GroEL/S. These findings suggest that the chaperonins have the potential to promote accumulation of genetic diversity.

How GroEL/S rescues mutant proteins with folding defects has been demonstrated in a series of studies by Teschke, King and colleagues [8–11]. Using a set of temperature-sensitive folding (*tsf*) mutants of the bacteriophage P22 coat protein, a model substrate which is amenable to detailed folding studies and has a clear functional assay, they have shown that *tsf* substitutions that are intolerable in the absence of GroEL/S chaperonins can be rescued in the presence of the chaperonins [8]. Such rescue occurs through direct interaction between the chaperonins and the destabilized protein, preventing aggregation monitored by decreased solubility [9]. Moreover, suppressor mutations in *tsf* mutants facilitate the chaperonin−substrate interaction [10], and chaperonins act to bias polypeptide flux away from aggregation of a folding intermediate and toward folding and assembly [10,11]. Interestingly, wild-type P22 coat protein is not a GroEL/S client, whereas its *tsf* mutants are [8,9].

## Rescuing Innovative Mutants

Although the P22 coat-protein work anticipates many more recent results, this system lacks a screen for functional diversification, leaving the possible functional effects of the additional mutations rescued by GroEL/S unaddressed. By contrast, enzymes have obvious diversification potential due to wide-ranging activities on different substrates. Focusing