The evolution and maintenance of gene clusters must involve recombination events. Studies on the segregation of *MAT* during meiosis in *C. neoformans* reveal that this locus is flanked by activators that increase recombination 10 to 50-fold above the genomic average [19]. These regions of activated recombination correlate with sequences with an increased G+C content, similar to γ class recombinational activators. These *MAT*-associated hotspots may play a central role in the evolutionary events outlined above that fashioned this unique genomic region [19]. Similar recombinational activators — γ, α or β hotspots, which recruit transcription factors or alter nucleosome positioning — may have played analogous roles in the evolution of biosynthetic, metabolic, and virulence-associated gene clusters. Thus, in addition to comparative genomics to understand gene cluster evolution, analysis of meiotic segregation patterns and DNA sequences near clusters provides a fertile avenue for investigation in *U. maydis* and other fungi.

In closing, the *U. maydis* genome sequence, associated expression patterns, and functional analysis of the 12 gene clusters [1] highlight the fact that novel host-microbe paradigms remain to be discovered and explored. These tools can reveal general principles by which hosts are subject to invasion and infection, and how gene clusters evolved and function as unique genomic structures.

References

1. Kämper, J., Kahmann, R., Bölker, M., Ma, L.-J., Brefort, T., Saville, B.J., Banuett, F., Kronstad, J.W., Gold, S.E., Müller, O., *et al*. (2006). Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. Nature *444*, 97–101.
2. Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.-R., Pan, H., *et al*. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature *434*, 980–986.
3. Cramer, R.A., Jr., Gamcsik, M.P., Brooking, R.M., Najvar, L.K., Kirkpatrick, W.R., Patterson, T.F., Balibar, C.J., Graybill, J.R., Perfect, J.R., Abraham, S.N., *et al*. (2006). Disruption of a nonribosomal peptide synthetase in *Aspergillus fumigatus* eliminates gliotoxin production. Eukaryot. Cell *5*, 972–980.
4. Gardiner, D.M., Cozijnsen, A.J., Wilson, L.M., Pedras, M.S.C., and Howlett, B.J. (2004). The sirodesmin biosynthetic gene cluster of the plant pathogenic fungus *Leptosphaeria maculans*. Mol. Microbiol. *53*, 1307–1318.
5. Kupfahl, C., Heinekamp, T., Geginat, G., Ruppert, T., Härtl, A., Hof, H., and Brakhage, A.A. (2006). Deletion of the *gliP* gene of *Aspergillus fumigatus* results in loss of gliotoxin production but has no effect on virulence of the fungus in a low-dose mouse infection model. Mol. Microbiol. *62*, 292–302.
6. Flor, H.H. (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. *28*, 275–296.
7. Catanzariti, A.-M., Dodds, P.N., Lawrence, G.J., Ayliffe, M.A., and Ellis, J.G. (2006). Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. Plant Cell *18*, 243–256.
8. Farfsing, J.W., Auffarth, K., and Basse, C.W. (2005). Identification of *cis*-active elements in *Ustilago maydis mig2* promoters conferring high-level activity during pathogenic growth in maize. Mol. Plant-Microbe Interact. *18*, 75–87.
9. Nierman, W.C., Pain, A., Anderson, M.J., Wortman, J.R., Kim, H.S., Arroyo, J., Berriman, M., Abe, K., Archer, D.B., Bermejo, C., *et al*. (2005). Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. Nature *438*, 1151–1156.
10. Bok, J.W., and Keller, N.P. (2004). LaeA, a regulator of secondary metabolism in *Aspergillus* spp. Eukaryot. Cell *3*, 527–535.
11. Gardiner, D.M., and Howlett, B.J. (2005). Bioinformatic and expression analysis of the putative gliotoxin biosynthetic gene cluster of *Aspergillus fumigatus*. FEMS Microbiol. Lett. *248*, 241–248.
12. Schirawski, J., Heinze, B., Wagenknecht, M., and Kahmann, R. (2005). Mating type loci of *Sporisorium reilianum*: novel pattern with three *a* and multiple *b* specificities. Eukaryot. Cell *4*, 1317–1327.
13. Fraser, J.A., Diezmann, S., Subaran, R.L., Allen, A., Lengeler, K.B., Dietrich, F.S., and Heitman, J. (2004). Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. PLoS Biol. *2*, e384.
14. del Poeta, M., Toffaletti, D.L., Rude, T.H., Sparks, S.D., Heitman, J., and Perfect, J.R. (1999). *Cryptococcus neoformans* differential gene expression detected *in vitro* and in vivo with green fluorescent protein. Infect. Immun. *67*, 1812–1820.
15. Fan, W., Kraus, P.R., Boily, M.-J., and Heitman, J. (2005). *Cryptococcus neoformans* gene expression during murine macrophages infection. Eukaryot. Cell *4*, 1420–1433.
16. Wong, S., and Wolfe, K.H. (2005). Birth of a metabolic gene cluster in yeast by adaptive gene relocation. Nat. Genet. *37*, 777–782.
17. Walton, J.D. (2000). Horizontal gene transfer and the evolution of secondary metabolite gene clusters in fungi: an hypothesis. Fungal Genet. Biol. *30*, 167–171.
18. Friesen, T.L., Stukenbrock, E.H., Liu, Z., Meinhardt, S., Ling, H., Faris, J.D., Rasmussen, J.B., Solomon, P.S., McDonald, B.A., and Oliver, R.P. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. Nat. Genet. *38*, 953–956.
19. Hsueh, Y.-P., Idnurm, A., and Heitman, J. (2006). Recombination hotspots flank the *Cryptococcus* mating-type locus: Implications for the evolution of a fungal sex chromosome. PLoS Genet. *2*, e184.
20. Gardiner, D.M., Waring, P., and Howlett, B.J. (2005). The epipolythiodioxopiperazine (ETP) class of fungal toxins: distribution, mode of action, functions and biosynthesis. Microbiology *151*, 1021–1032.

[1]School of Botany, The University of Melbourne, Victoria 3010, Australia.
[2]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710, USA.
E-mail: bhowlett@unimelb.edu.au, heitm001@duke.edu

DOI: 10.1016/j.cub.2006.11.047

Evolution: Lending a Helping Hand in Sperm Competition?

Most females mate with many males. This can be costly, but the benefits to females are often unclear. A new study raises the possibility that females could benefit through an unconventional genetic pathway, while also showing that males can inadvertently increase rival males' fitness in surprising ways.

Clarissa M. House, John Hunt and David J. Hosken

It has become increasingly clear that, in contrast with traditional views, females of most species mate with many males [1]. Understanding why they do this has become a mini-industry — mating is costly after all — and the consequences of female multiple-mating (polyandry) are often unclear. Explanations for polyandry are varied and range from the production of higher quality offspring — polyandry allows females to choose superior fathers for their offspring — to male

sexual-coercion — females mate multiply because males force them to. While the female benefits of polyandry remain hotly debated, one undisputed consequence of mating with multiple males is sperm competition, where the sperm of different males compete to fertilize a female's ova. Females could benefit by allowing sperm to compete if, for example, good sperm competitors father higher quality offspring [2], or produce sons that are themselves good at sperm competition [3].

Regardless of the female benefit, the male–male interactions that occur during this form of competition are viewed as unconditionally antagonistic: if one male does better, the other does worse. This fundamental premise is exemplified by the kamikaze-sperm hypothesis, which suggests males produce sperm especially designed to attack rival males' gametes [4], or by the idea that seminal fluid somehow recognises rival sperm and damages them [5], although there is little evidence for either of these scenarios [6,7]. The idea that competing males' ejaculates could interact in more synergistic ways, and that this also benefits females, is not one that has received any great attention, although this may change with recent findings published in *Current Biology* [8].

Working on the Australian field cricket (*Teleogryllus oceanicus*; Figure 1), a species already known for the positive effects of polyandry on embryo viability [9], García-Gonzáles and Simmons [8] found that males can enhance the viability of embryos sired by rivals. The protocol employed to come to this conclusion involved batches of six full-sisters that were grouped with two males unrelated to each other or the females. Each male mated twice to two females in a monogamous setting, so that there was no sperm competition; and then both males mated once each to the remaining two female — polyandrous matings, with sperm competition. Using a neutral morphological marker (eye colour) that enabled the paternity and survival of embryos to be assessed, the authors first documented embryo viability

Figure 1. Male (bottom) and female (top) Australian field crickets (*Teleogryllus oceanicus*) just after mating.

Photo courtesy of Francisco García-González.

differences between males in the monogamously mated sisters. Then, in the sperm competition experiment — where the females mated with the two males in succession — males with higher embryo viability competed against males producing embryos with lower viability.

If purely genetic effects determined embryo survival, males with low offspring viability in non-competitive matings should always produce low-viability embryos. What García-Gonzáles and Simmons [8] found in the competitive matings, however, was that males inducing high embryo viability in the non-competitive setting increased the viability of embryos sired by males previously shown to be poor embryo-viability enhancers. Furthermore, this effect was greatest when the difference in viability enhancement between the two males was greatest: when the viability enhancement of the two males was similar, embryo survival was occasionally reduced. The authors suggest that the females did not influence this pattern, as no significant female effects were detected. Furthermore, the proportion of eggs fertilized by a male was independent of his effect on embryo viability, indicating that females were not biasing paternity toward superior males. It therefore appears that the rescuing effect was not due to the mothers or to the direct action of the superior sires' genes, but instead rescue resulted from the quality of the environment provided by the male inducing high embryo viability. This opens up a whole new can of worms, raising the possibility that the benefits of polyandry may not necessarily have to be transmitted through conventional (additive) genetic pathways.

So how could this work? García-Gonzáles and Simmons [8] raise one intriguing possibility: indirect genetic effects. Individuals often have a strong impact on the environment for others, whether they be related, as in the case of mothers and offspring [10], or unrelated, as with sexual partners [11]. If there is variation in the quality of this environment and if this variation reflects genetic differences among individuals, then indirect genetic effects will exist and the environment will be heritable [12]. Previous work by García-Gonzáles and Simmons [13] has shown heritable differences between males in their ability to induce embryo viability. Moreover, a role for accessory gland products was implicated in this process through a positive genetic correlation between hatching

success and the weight of the accessory gland [13]. If the accessory gland products of high viability males do indeed provide the 'environment' for low viability males and are responsible for the observed rescuing effect in *T. oceanicus*, then indirect genetic effects may play an important role in the evolution of polyandry. At this early stage, however, the interpretation of indirect genetic effects should be treated with a degree of caution because the paternal effects documented could have primarily an environmental rather than a genetic basis.

Other questions, such as how the weight of the accessory gland relates to the composition of the substances it produces, and what other fitness consequences they may have also remain. Indeed, it has taken many years of extensive research to isolate some of the chemicals contained in *Drosophila* seminal fluid, and to understand some of their effects on both male and female fitness [14]. Why a few mating combinations reduced embryo viability while others increased it is also unclear, but this shows that, although the net effect across all matings was positive, embryo viability benefits to females through polyandry are not guaranteed. Furthermore, the lifetime fitness consequences of polyandry remain to be assessed in this cricket.

Irrespective of all these considerations, however, García-Gonzáles and Simmons [8] have made the obvious (yet novel) connection between the theory of indirect genetic effects and the interaction between males during sperm competition. The next logical step is to determine how indirect genetic effects influence the evolution of sperm competitiveness and the benefits that may be gained by polyandry. Theory indicates that indirect genetic effects can have important evolutionary consequences, including altering the rate and/or direction of evolution, generating large evolutionary time-lags in responses to selection, and even facilitating the evolution of traits with low levels of additive genetic variance [12].

Another recent study [15] has documented a broadly similar rescue effect in another insect, the pseudoscorpion *Cordylochernes scorpiodes*. Taking advantage of the fact that females in this species have a unique 'external-womb' form of viviparity that enables embryo viability to be directly monitored through development, these authors demonstrated that rates of embryo abortion were around 40% in brother–sister matings, and this was reduced considerably when females also mated with an unrelated male. Additionally, females mating exclusively with their brothers had fewer offspring in broods that did not abort.

At first glance, this finding appears consistent with previous studies showing biases in paternity towards unrelated males, and some of the most compelling evidence that polyandry is adaptive comes from studies demonstrating that multiple mating by females can defray the costs of inbreeding [16]. But paternity analysis showed this was not the case for the pseudoscorpions, with brothers actually siring disproportionately more offspring when mating in competition with unrelated males. Thus, the embryos of outbred males appear to rescue those of inbred males when developing together in a mixed-paternity brood. While there are a number of possible explanations for this observed 'rescuing' effect [17], the new study by Zeh and Zeh [15] clearly demonstrates the complex nature of the processes that determine how females can benefit from polyandry. Furthermore, like the cricket study, it highlights the fact that males may not always have only detrimental effects on each other during sperm competition, while also raising important questions about the generality of such findings.

The notion that interactions between individuals are important in post-copulatory sexual selection, including sperm competition, is not new. For example, studies [18] have shown that female genotypes influence fertilization success during sperm competition, and that a male's success in sperm competition depends on his competitor(s). In fact this is the basis of sexual selection at a very fundamental level — how good you are depends on the competition, and in a bar full of Brad Pitt look-alikes one's chances may be slim, while against a selection of Woody Allens, the odds are probably better. The García-Gonzáles and Simmons [8] study has elements of all the above, but the findings are very novel — it can pay to compete against top males, and female benefits from polyandry may be through indirect paternal effects — and the paternal effects documented are under appreciated. The interactions between rival males were not invariably positive, however, and as a result, females are not always certain to reap the rescue benefit.

Collectively, these studies [8,14] set a new precedent for studies of sperm competition and polyandry, and they highlight the importance of processes that occur during embryo development and how they have the potential to confound studies that measure paternity at hatching or birth (as has been previously noted [19]). Moreover, they both demonstrate that competing males may not always have purely adverse effects on each another. This raises the interesting possibility that inferior males may actively seek to mate after a competitively superior male in order to increase their net fitness, suggesting yet another route by which males could parasitise the ejaculates of their rivals, a possibility only recently formulated [20]. Finally, these new findings suggest the paths to fitness are many, and it seems, each is always trodden by some.

References

1. Birkhead, T.R., and Moller, A.P. (1998). Sperm Competition and Sexual Selection. (London: Academic Press).
2. Yasui, Y. (1997). A "good sperm" model can explain the evolution of costly multiple mating by females. Am. Nat. *149*, 573–584.
3. Radwan, J. (1998). Heritability of sperm competition success in the bulb mite, *Rhizoglyphus robini*. J. Evol. Biol. *11*, 321–327.
4. Bellis, M.A., Baker, R.R., and Gage, M.J.G. (1990). Variation in rat ejaculates consistent with the kamikaze-sperm hypothesis. J. Mamm. *71*, 479–480.
5. Harshman, L.G., and Prout, T. (1994). Sperm displacement without sperm transfer in *Drosophila melanogaster*. Evolution *48*, 758–766.

6. Snook, R.R., and Hosken, D.J. (2004). Sperm death and dumping in *Drosophila*. Nature *428*, 939–941.
7. Moore, H.D.M., Martin, M., and Birkhead, T.R. (1999). No evidence for killer sperm or other selective interactions between human spermatozoa in the ejaculates of different males in vitro. Proc. R. Soc. Lond. B *266*, 2343–2350.
8. Garcia-Gonzales, F., and Simmons, L.W. (2007). Paternal indirect genetic effects on offspring viability and the benefits of polyandry. Curr. Biol. *17*, 32–36.
9. Simmons, L.W. (2001). The evolution of polyandry: an examination of the genetic incompatibility and good-sperm hypothesis. J. Evol. Biol. *14*, 585–594.
10. Hunt, J., and Simmons, L.W. (2002). The genetics of maternal care: direct and indirect genetic effects in the dung beetle *Onthophagus Taurus*. Proc. Natl. Acad. Sci. USA *99*, 6828–6832.
11. Petfield, D., Chenoweth, S.F., Rundle, H.D., and Blows, M.W. (2005). Genetic variance in female condition predicts indirect genetic variance in male sexual display traits. Proc. Natl. Acad. Sci. USA *102*, 6045–6050.
12. Wolf, J.B., Brodie, E.D., III, Cheverud, J.M., Moore, A.J., and Wade, M.J. (1998). Evolutionary consequences of indirect genetic effects. Trends Ecol. Evol. *13*, 64–69.
13. Garcia-Gonzales, F., and Simmons, L.W. (2005). The evolution of polyandry: intrinsic sire effects contribute to embryo viability. J. Evol. Biol. *18*, 1097–1103.
14. Chapman, T. (2001). Seminal fluid mediated fitness traits in *Drosophila*. Heredity *87*, 511–521.
15. Zeh, J.A., and Zeh, D.W. (2006). Outbred embryos rescue inbred half-siblings in mixed paternity broods of live-bearing females. Nature *439*, 201–203.
16. Bretman, A., Wedell, N., and Tregenza, T. (2004). Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. Proc. Roy. Soc. Lond. B *271*, 159–164.
17. Hosken, D.J., and Tregenza, T. (2006). Evolution: inbreeding, multiple mating and embryonic aid. Curr. Biol. *16*, R202–R203.
18. Clark, A. (2002). Sperm competition and the maintenance of polymorphism. Heredity *88*, 148–153.
19. Gilchrist, A.S., and Partridge, L. (1997). Heritability of pre-adult viability differences can explain apparent heritability of sperm displacement ability in Drosophila melanogaster. Proc. Roy. Soc. Lond. B *264*, 1271–1275.
20. Hodgson, D.J., and Hosken, D.J. (2006). Sperm competition promotes the exploitation of rival ejaculates. J. Theor. Biol. *243*, 230–235.

Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall TR10 9EZ, UK.
E-mail: D.J.Hosken@exeter.ac.uk

DOI: 10.1016/j.cub.2007.01.017

Cell Division: Mid-Level Management

When a fission yeast cell divides, the anillin-like protein mid1p helps to position the contractile ring in the cell middle. Recent experiments from two groups have shown how the cell-polarity factor pom1p negatively regulates the distribution of mid1p.

Kenneth E. Sawin

Accurate partitioning of genomes during cell division requires not only high-fidelity chromosome segregation but also proper positioning of the actomyosin contractile ring that drives cytokinesis in animal and fungal cells. In animal cells, the position of the ring is determined by both positive and negative signals from the mitotic spindle (summarized in [1]), while in the budding yeast *Saccharomyces cerevisiae*, the ring forms at the site of bud emergence (see [2] for references). In the fission yeast *Schizosaccharomyces pombe*, which is rapidly becoming a popular model for quantitative studies of cytokinesis [3,4], the position of the cell nucleus positively and dynamically signals the future position of the contractile ring [5–7], via the protein mid1p [8,9]. New results [10,11] now indicate that negative signals also act to specify contractile ring position in fission yeast, and that these also operate via mid1p.

Mid1p was first identified from loss-of-function mutations in which the contractile ring is able to form, but its position is uncoupled from the position of the nucleus [8,9]. As a consequence of this, although *mid1* is not an essential gene *per se*, *mid1Δ* cells grow relatively poorly. Initial localization studies showed mid1p to be in the nucleus during interphase and at the plasma membrane during mitosis, first as spots within a broad ring in the cell middle and later as a tight ring, thus displaying some properties similar to the related protein anillin of higher eukaryotes (see [12] for references). Temporally, mid1p is one of the 'earliest' cortical factors known to be involved in contractile ring formation, and recent work has suggested that membrane-associated 'nodes' of mid1p may recruit myosin II and other proteins to the cell cortex, later coalescing to form a ring [3,13,14].

The movement of mid1p from nucleus to plasma membrane is thought to coordinate nuclear position with contractile ring placement. To do this accurately requires a dynamic localization of mid1p, and a key element of this scenario is that mid1p shuttles between nucleus and cytoplasm to become associated with the cortex. This has been borne out in experiments where both nuclear localization signals and nuclear export signals within mid1p have been manipulated [15].

While initial immunofluorescence observations using anti-mid1p antibodies showed a cortical membrane localization for mid1p only in mitosis, visualization of mid1–GFP fusions in living cells later revealed that mid1p is at the cortical membrane during interphase as well, specifically in the middle of cells, and this localization also follows the position of the nucleus [15]. (While GFP-tagged mid1 is functional and provides the basis for nearly all subsequent work, it should be noted that there is some evidence that it does not behave exactly like untagged mid1p [11,15,16].)

This sets the stage for the question: can a simple mechanism of shuttling from the nucleus to the membrane actually account for the interphase mid1p distribution seen *in vivo*, with a strong enrichment in the cell middle? In a collaboration between the Chang and Howard groups, Padte *et al.* [10] addressed this question by constructing an explicit mathematical model for