



# Creation and maintenance of variation in allorecognition loci: molecular analysis in various model systems

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Allorecognition is the ability of an organism to differentiate self or close relatives from unrelated conspecifics. Effective allorecognition systems are critical to the survival of organisms; they prevent inbreeding and facilitate fusions between close relatives. Where the loci governing allorecognition outcomes have been identified, the corresponding proteins often exhibit exceptional polymorphism. Two important questions about this polymorphism remain unresolved: how is it created, and how is it maintained. Because the genetic bases of several allorecognition systems have now been identified, including *alr1* and *alr2* in *Hydractinia*, fusion histocompatibility in *Botryllus*, the *het* (*vic*) loci in fungi, *tgrB1* and *tgrC1* in *Dictyostelium*, and self-incompatibility (SI) loci in several plant families, we are now poised to achieve a clearer understanding of how these loci evolve. In this review, we summarize what is currently known about the evolution of allorecognition loci, highlight open questions, and suggest future directions.

**Keywords:** allorecognition, mutation, recombination, variation, polymorphism, balancing selection

## INTRODUCTION

Allorecognition, the ability of an organism to differentiate self or close relatives from unrelated conspecifics, occurs throughout the tree of life (Buss, 1982), in anemones (Mercier et al., 2011), angiosperms (Allen and Hiscock, 2008), ascidians (Raftos, 1994; Saito et al., 1994; Harada et al., 2008), bacteria (Gibbs et al., 2008), bryozoans (Hughes et al., 2004), cellular slime molds (Shaalsky and Kessin, 2007), corals (Hidaka et al., 1997), fungi (Glass et al., 2000), gymnosperms (Pandey, 1960; Runions and Owens, 1998), hydroids (Grosberg et al., 1996), plasmodial slime molds (Clark, 2003), and sponges (Fernandez-Busquets and Burger, 1999).

Effective allorecognition systems are critical to the survival of organisms: the SI loci prevent inbreeding depression in plants, and in many colonial organisms, fusing to a closely related individual can provide competitive and reproductive advantages where space is limited and reproductive output is based on the size of the organism (Buss, 1982).

Despite decades of research, the genetic basis of allorecognition remains hidden in many groups, including bryozoans, corals, and sponges. Many marine invertebrate species are difficult to culture and breed, which limits the crossing experiments necessary to pinpoint genomic locations involved in allorecognition (Grosberg and Plachetzki, 2010).

However, researchers have been studying the SI (self-incompatibility) loci in angiosperms for some time. And allorecognition genes have recently been identified in ascidians (Fusion Histocompatibility, FuHC in *Botryllus schlosseri* and s-themis/v-themis in *Ciona intestinalis*; De Tomaso et al., 2005; Harada et al., 2008, reviewed in Ben-Shlomo, 2008), bacteria (Gibbs et al., 2008), cellular slime molds [*tgrB1* and *tgrC1* in *Dictyostelium discoideum* (Shaalsky and Kessin, 2007)], hydroids [*alr1* and *alr2* in *Hydractinia symbiolongicarpus* (Nicotra et al., 2009)], and fungi [*het* or *vic* loci (Glass et al., 2000)]. In this review we will focus only

on organisms where the genetic basis of allorecognition has been identified, and where the polymorphism in these loci has been studied. Although the loci governing self-incompatibility have recently been identified in the ascidian *C. intestinalis* (Harada et al., 2008) and the bacterium *Proteus mirabilis* (Gibbs et al., 2008), no evolutionary studies have yet been published.

In the systems where the loci governing allorecognition outcomes have been identified, the corresponding proteins have often exhibited exceptional polymorphism. In the clover *Trifolium pratense*, up to 193 S-alleles in the SI system were identified (Lawrence, 1996), and 13–16 S-alleles were identified from 20 *Arabidopsis lyrata* plants (Mable et al., 2003). In the colonial hydroid *H. symbiolongicarpus*, 35 alleles of the *alr2* allorecognition locus were sequenced from 18 colonies (Rosengarten et al., 2010).

Because allelic variation forms the basis of allorecognition, evolutionary studies of self/non-self recognition focus on this variation. If we can understand the evolutionary forces underlying the remarkable polymorphism in allorecognition loci, we gain valuable insights into the evolution and mechanisms of allorecognition systems. Two important questions about this polymorphism remain to be solved: how is it created and how is it maintained? We will address each question by summarizing and interpreting the available data.

## CREATION OF POLYMORPHISM

Mutation and recombination are the two processes that commonly create variation in allorecognition loci. These forces have been examined in several systems: *het/vic* loci in fungi, FuHC in *B. schlosseri*, SI loci in the Brassicaceae (*Arabidopsis* and *Brassica*) and Solanaceae (*Lycium*, *Petunia*, *Physalis*, *Solanum*), and *alr2* in *Hydractinia*.

In the fungus *Podospora anserina*, the *het-d* and *het-e* loci belong to a 10-member gene family; diversity is created by sharing

of WD-repeats through recombinational processes within and between loci in this family. Because *het-d* and *het-e* have a large copy number of repeats, mutations arise frequently in the WD-domains, also creating diversity. So polymorphism is created by plentiful mutations, which are then exchanged within and between loci, creating even more polymorphism. An accelerated mutational process called repeat induced polymorphism (RIP) that targets repeat sequences in fungi is thought to further generate variation (Paoletti et al., 2007). Recombination does not often occur within the A-mating type locus of the basidiomycete fungus *Coprinus cinereus* (Day, 1963). But infrequent recombination events have created diversity in this locus; this is unusually in sex-determining loci (May and Matzke, 1995). Researchers found reduced recombination near the mating type locus (MAT) in the chestnut blight fungus, *Cryphonectria parasitica* (Kubisiak and Milgroom, 2006).

In *B. schlosseri*, FuHC experiences a substantial amount of intragenic recombination, based on three independent measures:  $R_m$ , the correlation between physical distance and three measures of linkage disequilibrium, and levels of recombination across the protein (Nydam, Taylor and De Tomaso unpublished data). Six populations were examined, 112 alleles and 77 individuals for Exons 1–14, 111 alleles and 76 individuals for Exons 18–31. This data set was used for all *Botryllus* analyses discussed in this paper. The relative contributions of mutation and recombination in generating polymorphism in FuHC were determined by calculating  $\theta$  and  $R$  in DnaSP 5.10.01 (Librado and Rozas, 2009).  $\theta = 4^*N^*\mu$ , where  $N$  is the effective population size and  $\mu$  is the mutation rate per DNA sequence per generation.  $R = 4N^*r$  (Hudson, 1987), where  $N$  is the population size and  $r$  is the recombination rate per sequence.  $R$  is estimated from the variance of the average number of nucleotide differences between pairs of sequences (Hudson, 1987). All values were estimated using DnaSP. A ratio of  $\theta/R = 1$  signifies an equal contribution of mutation and recombination,  $>1$  a larger role for mutation, and  $<1$  a larger role for recombination.  $\theta/R$  was much less than one, so recombination clearly plays a larger role in the creation of FuHC than mutation.

When discussing the creation of variation in plant self-incompatibility (SI) loci, we must make a distinction between gametophytic and sporophytic SI systems. In gametophytic SI, the most common type of SI, the haploid self-incompatibility genotype of the pollen dictates its self-incompatibility phenotype. In sporophytic SI, the diploid self-incompatibility genotype of the plant dictates the self-incompatibility phenotype of the pollen produced by that plant (Newbigin et al., 1993).

Mutation plays a larger role than recombination in both gametophytic and sporophytic systems. Recombination would break up the association between pollen and pistil self-incompatibility loci, and thus is predicted to be suppressed around SI loci (Stein et al., 1991). Little evidence for recombination exists in gametophytic systems (Schierup et al., 2001), but this result may be due to the lack of power of recombination-detecting statistics, caused by the extraordinarily high polymorphism at these loci. Several successive mutations have occurred at the majority of segregating sites in these loci; this shows that mutation creates variation, but it also obscures the role of recombination. Recombination does play a substantial role in the creation of polymorphism in one species with gametophytic SI: *Petunia inflata* (Wang et al., 2001).

The authors state that recombination events must be rare, and that recombinant alleles causing a reduction in fitness are removed by natural selection.

Numerous tests in *Arabidopsis* sporophytic SI systems have yielded scant evidence for recombination (Kamau and Charlesworth, 2005; Charlesworth et al., 2006; Hagenblad et al., 2006; Edh et al., 2009). Recombination has been detected in SI loci of *Brassica* species (Kusaba et al., 1997; Awadalla and Charlesworth, 1999; Takuno et al., 2007) but only in genes or gene domains that do not play a direct role in self-incompatibility specificity (Takuno et al., 2007; Edh et al., 2009). Mutation must create the majority of variation at SI loci; multiple mutations at variable sites are well documented (Edh et al., 2009).

Recombination likely contributes to *alr2* polymorphism in *Hydractinia*, based on the discovery of chimeric alleles having regions characteristic of two distinct types of structural polymorphism (Rosengarten et al., 2010), but the relative contribution of mutation and recombination to allelic diversity has not been assessed.

Except in the cases of sex-determining loci and SI loci in plants (where recombination is suppressed), mutation and recombination interact in allorecognition systems to create polymorphism.

## MAINTENANCE OF POLYMORPHISM DISTRIBUTION OF POLYMORPHISM WITHIN AND AMONG POPULATIONS

Using the Analysis of Molecular Variance (AMOVA), evolutionary biologists routinely partition the total molecular variation in a particular gene into three mutually exclusive groups: among geographical regions, among populations within geographical regions, and within populations. For example, geographical regions could be Europe and North America: how much of the variation is found when comparing these two regions? The “among populations within geographical regions” category asks, within Europe, how much of the variation is found when comparing the Valencia (Spain), Bergen (Norway), and Lucerne (Switzerland) populations? And finally, how much variation is found when populations are examined individually? If a large portion of the variation is found within populations, this means there is little genetic differentiation between populations (e.g., the same alleles would be found in Valencia, Bergen, and Lucerne).  $F_{st}$  is a related statistic; a statistically significant  $F_{st}$  signifies genetic differentiation between populations in the data set, pairwise  $F_{st}$  statistics are used to determine whether any pair of populations is significantly differentiated.

AMOVA and  $F_{st}$  calculations can inform us about the evolutionary forces operating on the allorecognition loci, allowing us to understand how polymorphism is maintained. Comparing allorecognition loci to neutral loci (usually microsatellites) with respect to  $F_{st}$  values and percentage of polymorphism within vs. among populations (AMOVA) allows one to test whether selection is occurring. Loci experiencing balancing selection (which maintains variation) should have larger amounts of polymorphism within populations and smaller amounts among populations than neutral loci (assuming selection pressures are similar between populations), whereas the opposite pattern is expected for loci experiencing directional selection (Schierup et al., 2000). Similar

genetic differentiation between allorecognition loci and neutral loci is taken as evidence for neutral evolution (e.g., genetic drift).

One might assume directional selection is acting when significant population structure is recovered, but allelic variation at allorecognition loci is likely older than current population structure. Limited gene flow between populations (conserving ancient variation), rather than selection, could lead to differentiation between contemporary populations (Richman et al., 2003). Because of this, strong inferences of selection on allorecognition loci should only be made from AMOVA and *F<sub>st</sub>* values when these values are compared to other loci not presumed to be under balancing or directional selection.

AMOVA and *F<sub>st</sub>* analyses have been completed in three allorecognition systems: *het/vic* loci in fungi, FuHC in *B. schlosseri*, and SI loci in the Asteraceae (*Guizotia abyssinica*), and Brassicaceae (*Arabidopsis* and *Brassica*). In both the chestnut blight fungus (*C. parasitica*) and the dry rot fungus (*Serpula lacrymans*), *het/vic* loci lack significant genetic differentiation among populations (Milgroom and Cortesi, 1999; Kausarud et al., 2006). In the ascidian *B. schlosseri*, >90% of the variation in the FuHC gene is found within populations, and *F<sub>st</sub>* is not statistically significant (Nydam, Taylor, and De Tomaso unpublished data). This is in direct contrast to values obtained from two *B. schlosseri* genes not presumed to be under directional or balancing selection: mitochondrial cytochrome oxidase I and *vasa*. Both of these genes have less variation within populations than FuHC (81.2 and 27.16%, respectively), and both have highly significant *F<sub>st</sub>* values ( $p < 0.001$ ).

Patterns at SI loci are similar to those at *het/vic* and FuHC. In *G. abyssinica* (niger), 97% of the SI locus variation was found within populations, and *F<sub>st</sub>* values were very low (although statistically significant; Geleta and Bryngelsson, 2010). *F<sub>st</sub>* values are significantly lower when compared to neutral loci, in all cases (*A. lyrata*: Kamau et al., 2007; *A. halleri*: Ruggiero et al., 2008; *Brassica cretica*: Edh et al., 2009; *B. insularis*: Glemin et al., 2005). These results provide strong evidence for balancing selection driving the evolution of SI loci.

*Het/vic*, FuHC, and SI loci show similar patterns: a large percentage of the variation at these loci is found within populations, *F<sub>st</sub>* values are not often statistically significant, and *F<sub>st</sub>* values are significantly lower when compared with neutral loci. These patterns are consistent with a straightforward model of balancing selection, where selection pressures are similar in all environments.

#### TESTS OF SELECTION: POLYMORPHISM AND DIVERGENCE STATISTICS

Tests of selection using polymorphism (e.g., Tajima's *D*) and divergence (e.g.,  $d_N/d_S$ ) statistics commonly find evidence for selection at allorecognition loci. In fact, one of the earliest and most cited examples of  $d_N/d_S > 1$  (non-synonymous substitution rate greater than synonymous substitution rate) comes from the peptide-binding region (PBR) in mouse and human MHC (Hughes and Nei, 1988). For polymorphism statistics such as Tajima's *D* and Fu and Li's *D\** and *F\**, values statistically greater than zero are evidence for balancing selection, and less than zero for directional selection (Tajima, 1989; Fu and Li, 1993). A pattern of  $d_N/d_S > 1$  could indicate either directional or balancing selection (Garrigan and Hedrick, 2003); other

data must be examined to determine which type of selection is occurring.

We will describe all the available data from the less well-studied loci (*tgrB1* and *tgrC1* in *D. discoideum*, *alr2* in *H. symbiolongicarpus*, mating-compatibility genes and *het/vic* loci in fungus, and FuHC in *B. schlosseri*). A complete description of all relevant studies in the SI literature is beyond the scope of this review; we instead highlight several recent studies from this allorecognition system.

In the cellular slime mold *D. discoideum*, the genes *tgrB1* and *tgrC1* are involved in kin recognition. Certain sections of these genes have  $d_N/d_S$  ratios  $> 1$ ; the authors conclude that balancing selection is causing this pattern, given the extensive polymorphism at these loci (Benabentos et al., 2009). Nine codons in *alr2* of *H. symbiolongicarpus* have elevated  $d_N/d_S$  ratios; the majority are found in exon 2 (Rosengarten et al., 2010). The presence of 35 *alr2* alleles recovered from 36 individuals led the authors to conclude that negative frequency-dependent selection (a type of balancing selection where rare alleles are favored by selection) is occurring. At equilibrium, the alleles of a single locus subject to frequency-dependent selection are expected to be equally frequent (Grosberg, 1988).

Neither of two mating-compatibility genes examined in fungus species showed  $d_N/d_S > 1$  (May et al., 1999; Rau et al., 2007), but the *b1* mating type gene in the mushroom fungus *C. cinereus* was shown to be experiencing balancing selection by comparing the topologies of gene genealogies under balancing selection and neutral scenarios (May et al., 1999). *Het-c* in *Neurospora crassa* was determined to be evolving under balancing selection; evidence included trans-species polymorphisms and an increase in non-synonymous substitutions in and around the specificity region of *het-c* (Wu et al., 1998). Four codon positions of the WD-40 repeats in *het-d* and *het-e* of *P. anserina* have  $d_N/d_S > 1$ . The authors conclude that balancing selection, rather than directional selection, is operating, because of the high number of amino acid combinations at the four codons of interest (Paoletti et al., 2007).

FuHC in *B. schlosseri* experiences selection, based on both polymorphism and  $d_N/d_S$  statistics (Nydam, Taylor, and De Tomaso unpublished data). Values of polymorphism statistics (Tajima's *D*, Fu and Li's *D\** and *F\**) were significantly negative in all East Coast populations, as well as Monterey, CA, USA on the West Coast, consistent with directional selection. But negative polymorphism statistics could be due to selective or demographic processes (e.g., recent population growth). In the case of FuHC, the pattern is likely due to selection rather than demography, given that none of the polymorphism statistics were significantly negative for a housekeeping gene (*vasa*). 11 additional housekeeping genes are currently being sequenced, to confirm that demographic processes are not causing this pattern. Omega statistics pinpointed 24 codons throughout the protein have a greater than 95% probability of  $d_N/d_S > 1$ . Four exon groups contained clusters of these positively selected sites: Exons 5, 6, 20, and 27. Exons 5, 6, and 20 had significantly higher omega values than the rest of the gene for a subset of populations; these exons will be targeted in future functional studies. Other tests are being conducted to determine whether this pattern is due to balancing or directional selection.

Inference of selection at SI loci in plants begins with Sewall Wright, who wrote, "It also fairly obvious that selection would tend

to increase the frequency of any additional alleles that may appear.” (Wright, 1939). Because fertilization is aborted when pollen and pistil S-allele are identical, rare S-alleles have a selective advantage. As negative frequency-dependent selection is a type of balancing selection, researchers have spent considerable effort determining whether SI loci are evolving under balancing selection.

Data from numerous plant groups provide considerable support for balancing selection on SI loci. As in other allorecognition systems, the majority of these data are  $d_N/d_S$  ratios  $> 1$ . However,  $d_N/d_S$  ratios  $> 1$  are consistent with directional and balancing selection, so additional data are needed to determine which of these scenarios is occurring.  $d_N/d_S$  ratios  $> 1$  have been found in many plant families, both in gametophytic and sporophytic SI systems (Clark and Kao, 1991; Ishimizu et al., 1998; Sato et al., 2002; Takebayashi et al., 2003; Igic et al., 2007; Guo et al., 2011).  $d_N/d_S$  ratios  $> 1$  were corroborated with additional data to infer the action of balancing selection: significantly positive Tajima's  $D$  values, little population structure compared to neutral markers, and low recombination for *SRK* and *SCR* in *B. cretica* (Edh et al., 2009), trans-species polymorphisms in *SRK* and *SCR* in several *Arabidopsis* species (Sato et al., 2002; Guo et al., 2011).

## CONCLUSION

Unusually high polymorphism is a hallmark of allorecognition loci; how this polymorphism is created and maintained has interested biologists since Sewall Wright. From the studies presented in this review, we can conclude that polymorphism is created by an interaction between mutation and recombination, except in the cases of sex-determining loci and SI loci in plants (where recombination is suppressed).

AMOVA/Fst studies examining the distribution of polymorphism within and among populations in *Het/vic*, FuHC, and SI loci generally provide support for the role of balancing selection in maintaining polymorphism.

Divergence statistics often show patterns of  $d_N/d_S$  ratios  $> 1$  for allorecognition loci; these values are consistent with both directional and balancing selection. In many cases, additional evidence such as significantly positive polymorphism statistics and/or identification of trans-species polymorphisms provide support for balancing over directional selection in the maintenance of variation in allorecognition loci.

## REFERENCES

- Allen, A. M., and Hiscock, S. J. (2008). “Evolution and phylogeny of self-incompatibility systems in angiosperms” in *Self-Incompatibility in Flowering Plants – Evolution, Diversity and Mechanisms*, ed. V. E. Franklin-Tong (Berlin: Springer-Verlag), 73–101.
- Awadalla, P., and Charlesworth, D. (1999). Recombination and selection at *Brassica* self-incompatibility loci. *Genetics* 152, 413–425.
- Benabentos, R., Hirose, S., Sucgang, R., Curk, T., Katoh, M., Ostrowski, E., Strassmann, J., Queller, D., Zupan, B., Shaulsky, G., and Kuspa, A. (2009). Polymorphic members of the lag-gene family mediate kin-discrimination in *Dictyostelium*. *Curr. Biol.* 19, 567–572.
- Ben-Shlomo, R. (2008). The molecular basis of allorecognition in ascidians. *Bioessays* 30, 1048–1051.
- Buss, L. (1982). Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci. U.S.A.* 79, 5337–5341.
- Charlesworth, D., Kamau, E., Hagenblad, J., and Tang, T. L. (2006). Trans-specificity at loci near the self-incompatibility locus in *Arabidopsis*. *Genetics* 172, 2699–2707.
- Clark, A. G., and Kao, T.-H. (1991). Excess nonsynonymous substitution at shared polymorphic sites among self-incompatibility alleles of Solanaceae. *Proc. Natl. Acad. Sci. U.S.A.* 88, 9823–9827.
- Clark, J. (2003). Plasmodial incompatibility in the myxomycete *Didymium squamulosum*. *Mycologia* 95, 24–26.
- Day, P. R. (1963). The structure of the A mating-type factor in *Coprinus lagopus*: wild alleles. *Genet. Res.* 4, 323–325.
- De Tomaso, A. W., Nyholm, S. V., Palmeri, K. J., Ishizuka K. J., Ludington, W. B., Mitchel, K., and Weissman I. L. (2005). Isolation and characterization of a protochordate histocompatibility locus. *Nature* 438, 454–459.
- Edh, K., Widen, B., and Cepelitis, A. (2009). Molecular population genetics of the SRK and SCR Self-Incompatibility genes in the wild plant species *Brassica cretica* (Brassicaceae). *Genetics* 181, 985–995.
- Fernandez-Busquets, X., and Burger, M. M. (1999). Cell adhesion and histocompatibility in sponges. *Microsc. Res. Tech.* 44, 204–218.
- Fu, Y. X., and Li, W. H. (1993). Statistical tests of neutrality of mutations. *Genetics* 133, 693–709.
- Garrigan, D., and Hedrick, P. W. (2003). Detecting adaptive molecular polymorphism: lessons from the MHC. *Evolution* 57, 1707–1722.

## FUTURE DIRECTIONS

We have only identified the loci involved in a handful of allorecognition systems, and an obvious future direction lies in identifying allorecognition loci in anemones, bryozoans, corals, plasmodial slime molds, and sponges. Such discoveries require painstaking work, and in some cases are hindered by the biology of the organisms. So while this may be a long term goal, it is nevertheless an important one if we are to achieve an understanding of the evolution of allorecognition across the tree of life.

A shorter term direction for evolutionary studies of allorecognition should involve the loci that have recently been discovered. We could not locate information on the distribution of polymorphism within and among populations for *tgrB1/tgrC1* in *D. discoideum*, or *alr2* in *H. symbiolongicarpus*, or on the creation of polymorphism for *tgrB1/tgrC1* in *D. discoideum*. No evolutionary studies have been conducted on the self-incompatibility genes in *C. intestinalis*. Additionally, recent studies concluding that balancing selection is occurring based on divergence statistics and the presence of polymorphism could provide more data to support these conclusions, including evidence of trans-species polymorphisms and AMOVA/Fst analyses showing larger amounts of polymorphism within populations and smaller amounts among populations than neutral loci. And finally, we have some information on the specific type of balancing selection operating in a well-studied allorecognition system (negative frequency-dependent selection in SI loci). Further analyses of the newly discovered loci should uncover more specifics about the types of balancing selection maintaining variation at these loci.

In well-studied allorecognition systems like the SI loci in plants, we have information on the evolution of allorecognition loci in many species. This allows us to examine the evolution of allorecognition across large phylogenetic groups, enabling broader conclusions than would be possible if only model organisms were used. Allorecognition loci can now be more easily identified and studied in species closely related to *B. schlosseri*, *C. intestinalis*, *D. discoideum*, and *H. symbiolongicarpus*. Using this approach, we could begin to understand the evolution of allorecognition not just in *B. schlosseri* and *C. intestinalis*, but in the urochordates as a whole, not just in *D. discoideum* but in the social amoebae, not just in *H. symbiolongicarpus* but in hydroids generally.

- Geleta, M., and Bryngelsson, T. (2010). Population genetics of self-incompatibility and developing self-compatible genotypes in niger (*Guizotia abyssinica*). *Euphytica* 176, 417–430.
- Gibbs, K. A., Urbanowski, M. L., and Greenberg, E. P. (2008). Genetic determinants of self identity and social recognition in bacteria. *Science* 321, 256–259.
- Glass, N. L., Jacobson, D. J., and Shiu, P. K. (2000). The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. *Annu. Rev. Genet.* 34, 165–186.
- Glemin, S., Gaude, T., Guillemin, M.-L., Lourmas, M., Olivieri, E., and Mignot, A. (2005). Balancing selection in the wild: testing population genetics theory of self-incompatibility in the rare species *Brassica insularis*. *Genetics* 171, 279–289.
- Grosberg, R. K. (1988). The evolution of allorecognition specificity in clonal invertebrates. *Q. Rev. Biol.* 63, 377–412.
- Grosberg, R. K., Levitan, D. R., and Cameron, B. B. (1996). Evolutionary genetics of allorecognition in the colonial hydroid *Hydractinia symbiolongicarpus*. *Evolution* 50, 2221–2240.
- Grosberg, R. K., and Plachetzki, D. (2010). “Marine invertebrates: genetics of colony recognition,” in *Encyclopedia of Animal Behavior*, eds M. D. Breed and J. Moore (Oxford: Academic Press), 381–388.
- Guo, Y.-L., Zhao, X., Lanz, C., and Weigel, D. (2011). Evolution of the S-locus region in *Arabidopsis thaliana* relatives. *Plant Physiol.* 157, 937–946.
- Hagenblad, J., Bechsgaard, J., and Charlesworth, D. (2006). Linkage disequilibrium between incompatibility locus region genes in the plant *Arabidopsis lyrata*. *Genetics* 173, 1057–1073.
- Harada, Y., Takagaki, Y., Sunagawa, M., Saito, H., Yamada, L., Taniguchi, H., Shoguchi, E., and Sawada, H. (2008). Mechanism of self sterility in a hermaphroditic chordate. *Science* 320, 548–550.
- Hidaka, M., Yurugi, K., Sunagawa, S., and Kinzie, R. A. III. (1997). Contact reactions between young colonies of the coral *Pocillopora damicornis*. *Coral Reefs* 16, 13–20.
- Hudson, R. R. (1987). Estimating the recombination parameter of a finite population model without selection. *Genet. Res.* 50, 245–250.
- Hughes, A. L., and Nei, M. (1988). Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature* 335, 167–170.
- Hughes, R. N., Manriquez, P. H., Morley, S., Craig, S. F., and Bishop, J. D. D. (2004). Kin or self-recognition? Colonial fusibility of the bryozoan *Celleporella hyalina*. *Evol. Dev.* 6, 431–437.
- Igic, B., Smith, W. A., Robertson, K. A., Schaal, B. A., and Kohn, J. R. (2007). Studies of self-incompatibility in wild tomatoes: I. S-allele diversity of *Solanum chilense* Dun. (Solanaceae). *Heredity* 99, 553–561.
- Ishimizu, T., Endo, T., Yamaguchi-Kabata, Y., Nakamura, K. T., Sakiyama, F., and Norioka, S. (1998). Identification of regions in which positive selection may operate in S-RNase of Rosaceae: implication for S-allele-specific recognition sites in S-RNase. *FEBS Lett.* 440, 337–342.
- Kamau, E., Charlesworth, B., and Charlesworth, D. (2007). Linkage disequilibrium and recombination rate estimates in the self-incompatibility region of *Arabidopsis lyrata*. *Genetics* 176, 2357–2369.
- Kamau, E., and Charlesworth, D. (2005). Balancing selection and low recombination affect diversity near the self-incompatibility loci of the plant *Arabidopsis lyrata*. *Curr. Biol.* 15, 1773–1778.
- Kausserud, H., Saetre, G.-P., Schmidt, O., Decock, C., and Schumacher, T. (2006). Genetics of self/nonself recognition in *Serpula lacrymans*. *Fungal Genet. Biol.* 43, 503–510.
- Kubisiak, T. L., and Milgroom, M. G. (2006). Markers linked to vegetative incompatibility (vic) genes and a region of high heterogeneity and reduced recombination near the mating type locus (MAT) in *Cryphonectria parasitica*. *Fungal Genet. Biol.* 43, 453–463.
- Kusaba, M., Nishio, T., Satta, Y., Hinata, K., and Ockendon, D. (1997). Striking sequence similarity in inter- and intra-specific comparisons of class I SLG alleles from *Brassica oleracea* and *Brassica campestris*: implications for the evolution and recognition mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 94, 7673–7678.
- Lawrence, M. J. (1996). Number of incompatibility alleles in clover and other species. *Heredity* 76, 610–615.
- Librado, P., and Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452.
- Mable, B. K., Schierup, M. H., and Charlesworth, D. (2003). Estimating the number, frequency, and dominance of S-alleles in a natural population of *Arabidopsis lyrata* (Brassicaceae) with sporophytic control of self-incompatibility. *Heredity* 90, 422–431.
- May, G., and Matzke, E. (1995). Recombination and variation at the A mating-type of *Coprinus cinereus*. *Mol. Biol. Evol.* 12, 794–802.
- May, G., Shaw, F., Badrane, H., and Vekemans, H. (1999). The signature of balancing selection: fungal mating compatibility gene evolution. *Proc. Natl. Acad. Sci. U.S.A.* 96, 9172–9177.
- Mercier, A., Sun, Z., and Hamel, J.-F. (2011). Internal brooding favours pre-metamorphic chimerism in a non-colonial cnidarian, the sea anemone *Urticina felina*. *Proc. Biol. Sci.* 278, 3517–3522.
- Milgroom, M. G., and Cortesi, P. (1999). Analysis of population structure of the chestnut blight fungus based on vegetative incompatibility genotypes. *Proc. Natl. Acad. Sci. U.S.A.* 96, 10518–10523.
- Newbigin, E., Anderson, M. A., and Clarke, A. E. (1993). Gametophytic self-incompatibility systems. *Plant Cell* 5, 1315–1324.
- Nicotra, M. L., Powell, A. E., Rosengarten, R. D., Moreno, M., Grimwood, J., Lakkis, F. G., Dellaporta, S. L., and Buss, L. W. (2009). A hypervariable invertebrate allodeterminant. *Curr. Biol.* 19, 583–589.
- Pandey, K. K. (1960). Incompatibility in *Abutilon* ‘hybridum.’ *Am. J. Bot.* 47, 877–883.
- Paoletti, M., Saupe, S. J., and Clave, C. (2007). Genesis of a fungal non-self recognition repertoire. *PLoS ONE* 3, e283. doi:10.1371/journal.pone.0000283
- Raftos, D. (1994). Allorecognition and humoral immunity and tunicates. *Ann. N. Y. Acad. Sci.* 712, 227–244.
- Rau, D., Attene, G., Brown, A. H. D., Nanni, L., Maier, F. J., Balmas, V., Saba, E., Schafer, W., and Papa, R. (2007). Phylogeny and evolution of mating-type genes from *Pyrenophora teres*, the causal agent of barley “net blotch” disease. *Curr. Genet.* 51, 377–392.
- Rosengarten, R. D., Moreno, M. A., Lakkis, F. G., Buss, L. W., and Dellaporta, S. L. (2010). Genetic diversity of the allodeterminant *alr2* in *Hydractinia symbiolongicarpus*. *Mol. Biol. Evol.* 28, 933–947.
- Richman, A. D., Gerardo Herrera, L., Nash, D., and Schierup, M. K. (2003). Relative roles of mutation and recombination in generating allelic polymorphism at an MHC class II locus in *Peromyscus maniculatus*. *Genet. Res.* 82, 89–99.
- Ruggiero, M. V., Jacquemin, B., Castric, V., and Vekemans, X. (2008). Hitchhiking to a locus under balancing selection: high sequence diversity and low population subdivision at the S-locus genomic region in *Arabidopsis halleri*. *Genet. Res.* 90, 37–46.
- Runions, C. J., and Owens, J. N. (1998). “Evidence of pre-zygotic self-incompatibility in a conifer,” in *Reproductive Biology*, eds S. J. Owens and P. J. Rudall (Kew: Royal Botanic Gardens), 255–264.
- Saito, Y., Hirose, E., and Watanabe, H. (1994). Allorecognition in compound ascidians. *Int. J. Dev. Biol.* 38, 237–247.
- Sato, K., Nishio, T., Kimura, R., Kusaba, M., Suzuki, T., Hatakeyama, K., Ockendon, D. J., and Satta, Y. (2002). Coevolution of the S-Locus genes SRK, SLG, and SP11/SCR in *Brassica oleracea* and *B. rapa*. *Genetics* 162, 931–940.
- Schierup, M. H., Mikkelsen, A. M., and Hein, J. (2001). Recombination, balancing selection, and phylogenies in MHC and self-incompatibility genes. *Genetics* 159, 1833–1844.
- Schierup, M. H., Vekemans, X., and Charlesworth, D. (2000). The effect of subdivision on variation at multi-allelic loci under balancing selection. *Genet. Res.* 76, 51–62.
- Shaulsky, G., and Kessin, R. (2007). The cold war of the social amoebae. *Curr. Biol.* 17, 684–692.
- Stein, J., Howlett, B., Boyes, D. C., Nasrallah, M. E., and Nasrallah, J. B. (1991). Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. *Proc. Natl. Acad. Sci. U.S.A.* 88, 8816–8820.
- Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595.
- Takebayashi, N., Brewer, P. B., Newbigin, E., and Uyenoyama, M. K. (2003). Patterns of variation within self-incompatibility loci. *Mol. Biol. Evol.* 20, 1778–1794.
- Takuno, S., Fujimoto, R., Sugimura, T., Sato, K., Okamoto, S., Zhang, S.-L., and Nishio, T. (2007). Effects of recombination on hitchhiking

- diversity in the *Brassica* self-incompatibility locus complex. *Genetics* 177, 949–958.
- Wang, X., Hughes, A. L., Tsukamoto, T., Ando, T., and Kao, T.-H. (2001). Evidence that intragenic recombination contributes to allelic diversity of the S-RNase gene at the self-incompatibility (S) locus in *Petunia inflata*. *Plant Physiol.* 125, 1012–1022.
- Wright, S. (1939). The distribution of self-sterility alleles in populations. *Genetics* 24, 538–552.
- Wu, J., Saupe, S. J., and Glass, N. L. (1998). Evidence for balancing selection operating at the het-c heterokaryon incompatibility locus in a group of filamentous fungi. *Proc. Natl. Acad. Sci. U.S.A.* 95, 12398–12403.
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