Letter to the Editor

Is Allorecognition Specificity in *Hydractinia symbiolongicarpus* Controlled by a Single Gene?

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Manuscript received August 23, 1996
Accepted for publication November 12, 1996

IN many benthic, colonial invertebrates, the capacity to distinguish conspecific self from nonself, or allorecognition, plays a crucial role in determining the nature and outcome of somatic, and perhaps gametic, interactions (BUSS 1982, 1990; GROSBERG 1988). One of the most striking features of invertebrate allorecognition systems is the extraordinary precision with which self can be distinguished from nonself, even in very large populations. To the extent that allorecognition specificity is heritable, this precision suggests that populations of colonial invertebrates carry unusually high levels of genetic variation, rivalling levels found at MHC Class I and II loci (*e.g.*, POTTS and WAKELAND 1990; PARHAM and OHTA 1996) and self-incompatibility loci in angiosperms (reviewed in CHARLESWORTH 1995). However, unlike the vertebrate major histocompatibility complex, or many plant self-incompatibility systems, the question of whether one or a few loci (with high levels of allelic diversity) or multiple loci (with more modest amounts of variation per locus) control invertebrate allorecognition remains largely unexplored.

Some of the first attempts to decipher the formal genetics of invertebrate allorecognition met with considerable success. For example, among ascidians in the genera Botryllus and Botrylloides, a single locus with multiple codominant alleles appears to control the acute fusion/rejection response; juxtaposed individuals sharing one or both alleles at this locus somatically fuse, whereas those not sharing an allele reject each other (OKA and WATANABE 1957; SABBADIN 1962; SCOFIELD *et al.* 1982; YUND and FELDGARDEN 1992). However, comparable studies of cnidarians, especially on members of the genus Hydractinia, yielded inheritance patterns that did not conform to the predictions of the simple Botryllus genetic model (CROWELL 1950; HAUENSCHILD 1954, 1956; MÜLLER 1964; IVKER 1972; PASQUIER 1974). In a recent paper, MOKADY and BUSS (1996) used an inbred strain of *H. symbiolongicarpus* to reexamine the transmission genetics of allorecognition specificity in this species and surprisingly concluded that, "The results are in complete agreement with a model of *Hydractinia* allorecognition as a one-locus trait, with co-dominant expression of alleles, such that one shared allele yields a fusible phenotype."

In this paper, we first show that a simple and powerful prediction of the one-locus model proposed by MOKADY and BUSS (1996) does not withstand an empirical test. This raises the question of how MOKADY and BUSS (1996) reached the conclusion that a single locus controls allorecognition specificity in *H. symbiolongicarpus.* To address this question, we evaluate the limitations of the mating scheme described in their study, which could potentially bias the results in favor of the preferred hypothesis. Second, we show that their sample sizes are too small to distinguish among the predictions generated by other simple and plausible models of inheritance, leading to a high probability of Type II statistical errors. We then assess the rationale they used to dismiss the operation of a multi-locus system. Finally, we evaluate the merits of their proposition that modifier loci, whose effects were presumably minimized by their mating design, can alter observed fusion/rejection frequencies, such that the inheritance of a Botryllus-like system appears to be more complicated than it really is. Although their data are consistent with a Botryllus-like model of inheritance of allorecognition specificity in *H. symbiolongicarpus,* their analysis does not provide a decisive test of this model.

A simple test of the one-locus model: Assume that a single locus, A, with multiple codominant alleles, $A_{1,2,3\ldots}$, controls the allorecognition response in Hydractinia, as it does in Botryllus. Individuals sharing one or both alleles at the locus are compatible (*i.e.*, they fuse); those sharing neither allele are incompatible (*i.e.*, they reject). With these matching criteria, at least 200 equally frequent alleles would have to be segregating to account for the high frequencies of incompatibility (95–100% rejections) observed in tests among members of wild populations (CURTIS *et al.* 1982). Consequently, most individuals in the population will be heterozygous for different alleles at the A locus. If two such individuals mated (*e.g.*, $A_1A_2 \times A_3A_4$), the F_1 progeny would fall into one of four equally frequent genotypic

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classes: A_1A_3, A_1A_4, A_2A_3, A_2A_4. Each of these genotypes would be compatible with members of its own class, plus two out of the remaining three others. Thus, in such a mating 75% of full-sib pairs should fuse, and 25% should reject. Similarly, fusion/rejection frequencies should be 50:50 among half-sibs (provided that all three parents carry different alleles).

MOKADY and BUSS (1996) did not report data for compatibility frequencies among full-sibs and half-sibs. However, in GROSBERG *et al.* (1996) two of us explicitly tested these predictions. In nine full-sibships bred from field-collected pairs, rejection frequencies consistently approached 50% (mean = 47.2%; upper 95% CL = 50.7%; lower 95% CL = 43.7%), a value that significantly exceeds the predicted 25%. Similarly in two half-sibships, the rejection frequencies were 74.0% (n = 100; upper 95% CL = 82.3%; lower 95% CL = 64.3%) and 57.0% (n = 100; upper 95% CL = 66.7%; lower 95% CL = 46.7%). These discrepancies alone compel us to reject the Botryllus model insofar as it accounts for the inheritance of allorecognition specificity in *H. symbiolongicarpus.* Contrary to the conclusion of MOKADY and BUSS (1996), there must be alleles segregating at more than a single allorecognition locus in *H. symbiolongicarpus.*

The mating scheme: In an attempt to resolve the ambiguities raised in previous studies of the transmission genetics of allorecognition specificity in Hydractinia and, in particular, to minimize the effects of variation in genetic background on the expression of allorecognition specificity, MOKADY and BUSS (1996) first bred a reporter strain (their *G* line) through at least six generations of brother-sister matings, using only fusible pairs. They established Lineage A from a mating between an individual from the reporter strain and a single field-collected individual (α). With the F_1 progeny from this mating, they initiated a series of incrosses, intercrosses, and backcrosses (schematized in their Figure 1), and counted fusion/rejection frequencies between offspring from each of these crosses and members of the reporter lineage. They then compared their observed frequencies to those predicted under a Botryllus-like model of inheritance. They also generated another sibship of F_1 progeny by mating a member of their reporter line to another field-collected individual (Lineage B). They tested the compatibility response of three of these offspring to F_3's from Lineage A.

Unless there truly is a single, highly polymorphic locus controlling allorecognition specificity in *H. symbiolongicarpus,* such a mating scheme could underestimate (but definitely not overestimate) the actual number of loci segregating in the population. Regardless of the true underlying genetic system controlling allorecognition, their mating design, at best, could only be used to estimate the number of loci segregating in the α individual. For example, with four equally frequent alleles at a locus, on average the locus will not be segregating in one of four members of the population. With the fusion criteria assumed by MOKADY and BUSS (1996), five allorecognition loci, each carrying four equally frequent alleles, could account for the frequencies of fusion they reported among field-collected colonies. If this were the correct model, it is quite possible that fewer than five loci would be segregating in the single field-collected individual used to initiate Lineage A. [Note that MOKADY and BUSS (1996) assumed that the reporter strain was not segregating for the trait: "the lineage was deemed homozygous for fusibility."] In general, the magnitude of the underestimate will depend on the number of allorecognition loci, the numbers and frequencies of alleles per locus, and the number of progeny assayed for the segregation of the trait. The underestimate could be small if the number of allorecognition loci ($\approx$5) and number of alleles per locus ($\approx$5–7) were as large as estimated by GROSBERG *et al.* (1996). In any case, the use of a single inbred line can bias the results in the direction of a single-locus model.

Type II error: To test critically a genetic model for the inheritance of any trait, it is essential not only that the data be consistent with the predictions of the model, but also that they be inconsistent with credible alternatives. MOKADY and BUSS (1996) accept a one-locus model for the inheritance of allorecognition specificity in *H. symbiolongicarpus* based largely on the consistency of their data with the predictions of this model. We calculated the upper and lower 95% confidence intervals of their estimates of fusion/rejection frequencies for all six crosses in which expected frequencies differed from 100% fusions or rejections (Table 1). Inspection of this table reveals that in many cases, the number of compatibility tests is so small that most alternative models could not be rejected, even those making very different predictions about fusion/rejection frequencies. Even in their best case (Cross F_2; n = 34), the one-locus model predicts 75% fusions and 25% rejections to the reporter strain. They observed 76% fusions; however, the lower and upper 95% confidence intervals on this estimate range from 59% to 89%. After obtaining a result consistent with the predictions of the Botryllus model, MOKADY and BUSS (1996) performed many fewer compatibility tests in subsequent crosses and backcrosses (rather than performing more compatibility tests, to improve the statistical rigor of their tests). For example, in the F_2 backcross BC-3, the one-locus model predicts 50% fusions and 50% rejections. MOKADY and BUSS (1996) reported that $^3/_8$ tests yielded fusion, consistent with the 50:50 expectation, but the 95% confidence intervals include values ranging from 9% through 76%. Thus, while we agree that these data are consistent with the predictions of a one-locus model, the data lack the statistical power both to reject the proposed model, as well as to distinguish the predictions of this model from those involving different numbers of loci, alleles per locus, and fusibility criteria.

The argument against polygenic control: At its simplest, three attributes of a genetically based allorecognition system ultimately determine compatibility frequen-

TABLE 1

Expected fusion frequencies, observed fusion frequencies, and upper and lower 95% binomial confidence intervals based on the fusibility data reported in Table 1 of MOKADY and BUSS (1996)

	Expected fusion[b]	Observed fusion	n	Lower 95%CI	Upper 95%CI
Cross[a]					
F_2	0.75	0.76	34	0.59	0.89
F_3					
B	0.75	0.74	23	0.52	0.90
C	0.50	0.67	18	0.41	0.87
Backcrosses					
F_1					
BC2	0.50	0.62	13	0.32	0.86
BC3	0.75	0.77	13	0.46	0.95
BC5	0.50	0.38	8	0.09	0.76

[a] Notation follows MOKADY and BUSS (1996).

[b] Expected fusion frequencies of progeny from the designated cross to the "reporter" strain of MOKADY and BUSS (1996), under the assumptions that (1) one locus, with codominant alleles, controls allorecognition, and (2) individuals sharing one or both alleles at the locus will fuse.

cies: the number of loci controlling specificity, the number of alleles per locus, and the allelic matching criteria that stipulate the level and kind of allelic disparity or similarity that elicit fusion or rejection (GROSBERG *et al.* 1996). Depending upon the matching criteria, a variety of relatively simple genetic systems, ranging from a single locus with hundreds of alleles, through a few loci (*i.e.*, three to five) with 10 or fewer alleles per locus, to hundreds of loci with as few as two alleles per locus, could produce the extremely low rates of compatibility usually observed in natural populations of colonial marine invertebrates (CURTIS *et al.* 1982).

MOKADY and BUSS (1996) argue against a multilocus system from two perspectives. The first rests on the consistency of their data with the predictions of a Botryllus-like model and the inconsistency of their findings with other credible models. We showed above that this consistency is weak. The second line of argument in favor of unifactorial inheritance requires rejection of multilocus models [see Table 3 in MOKADY and BUSS (1996)]. The tests presented in Table 3 of MOKADY and BUSS (1996) explicitly make the highly restrictive assumptions that (1) the reporter strain is homozygous at all allorecognition loci and (2) 50% or 100% of alleles must be shared at each locus to generate intergenotypic fusion. More importantly, but unstated, the analysis implicitly assumes that (3) there are only two alleles per locus segregating in the population. Thus, in developing this second line of argument, MOKADY and BUSS (1996) make several critical simplifying assumptions that bias the tests in favor of rejecting multilocus control. If these assumptions were relaxed, especially assumptions 2 and 3, then the data in MOKADY and BUSS (1996) could be explained by any number of rather simple polygenic systems that they did not evaluate (CURTIS *et al.* 1982; GROSBERG *et al.* 1996).

The second assumption presumably derives from knowledge of fusibility criteria in Botryllus (OKA and WATANABE 1957; SABBADIN 1962; SCOFIELD *et al.* 1982). However, there is no reason to suppose that this is the only reasonable set of matching rules. For instance, the total number of alleles shared by two individuals will be governed by the number of loci and numbers of alleles per locus segregating in the population, as well as their relatedness. In a polygenic system, individuals could fuse even if they shared fewer than a single allele per locus (or reject if they shared more than a single allele per locus). These fusion criteria depend on the underlying physiological and molecular bases of allorecognition, which are not yet described in detail for any colonial invertebrate.

We know of no evidence supporting the third assumption that multilocus invertebrate allorecognition systems are somehow limited to a maximum of two alleles per locus. Of course, as the number of alleles per locus increases, so, too, does the potential precision of the allorecognition system. This will, in turn, decrease the number of loci that must be segregating to confer a given level of specificity.

Their data yield few insights into which of these alternatives is the most likely, in part because MOKADY and BUSS (1996) inferred parental genotypes in many of their crosses based on the observed fusion/rejection frequencies of offspring to the reporter strain under the assumptions of the single-locus Botryllus model. With even a slightly more complex genetic system, it would be nearly impossible to infer parental genotypes from such data, especially given the broad confidence intervals on their estimates of compatibility frequencies.

The appeal to modifier loci: MOKADY and BUSS (1996) proposed that the undetected segregation of modifier loci, whose effect should have been minimized by their mating design, could account for the failures of others to identify a single locus that controls allorecognition specificity: "While our findings collectively establish a monofactorial control of fusibility in *Hydrac-*

tinia, we would still expect subsequent work with these or other lineages to reveal modifiers of the expression of this locus." MOKADY and BUSS (1996) do not specify the effects of such loci, except to imply that the actions of such loci could account for discrepancies between the predictions of the Botryllus model and previously reported patterns of fusion/rejection. In principle, multiple minor genes could nonadditively interact with alleles at a major allorecognition locus to determine the outcome of tissue interactions between colonies.

The mating scheme used by MOKADY and BUSS (1996) to generate lineage A may have minimized the possibility that modifiers segregating in the population as a whole found their way into this lineage. However, the same limitations of the data reported by MOKADY and BUSS (1996), which make it difficult to reject a one-locus model of inheritance, also make it difficult to determine whether the failure to detect modifiers is due to the absence of modifiers only among the members of Lineage A (descended from a single cross), or their absence from the population as a whole.

Unfortunately, there is no *positive,* direct evidence for the segregation of modifiers with nonadditive effects in any study of the genetics of allorecognition in Hydractinia. For example, if modifiers were segregating in populations of *H. symbiolongicarpus,* their effects on fusion/rejection frequencies should be apparent in at least some matings. The results presented in GROSBERG *et al.* (1996) and summarized above show highly consistent compatibility frequencies among full-sib progeny from nine crosses between field-collected colonies. Such consistency is unexpected if modifiers were segregating in the population. In the absence of direct evidence for epistasis or other forms of nonadditivity, a model that incorporates the segregation of hypothetical modifier loci differs only semantically from an additive polygenic model.

Conclusions and prospects: For the progeny derived from the single cross between the α field-collected strain and the reporter strain (and subsequent crosses using these F_1's), the compatibility data reported by MOKADY and BUSS (1996) are consistent with a broad spectrum of genetic models, including the hypothesis that a one-locus system governs the inheritance of allorecognition specificity in *H. symbiolongicarpus.* The limitations of their mating scheme and the weak statistical power of the data caution against specifying which of these models best accounts for fusion/rejection frequencies among progeny ultimately derived from a single cross, much less generalizing to the number of loci segregating at the level of the population. Most importantly, observed fusion/rejection frequencies among full- and half-sibs *consistently* fail to match the predictions of a simple one-locus model. Thus, we conclude that although MOKADY and BUSS (1996) have shown that at least one locus segregates for allorecognition specificity (*i.e.,* allorecognition specificity has a heritable component) in populations of *H. symbiolongicarpus,* their data alone are not sufficient to distinguish among alternative models for the transmission genetics of allorecognition specificity in this species. We propose instead that their findings are consistent with a number of relatively simple multilocus models (analyzed in GROSBERG *et al.* 1996) that more fully account for what is currently known about the segregation of allorecognition specificity in half-sibships, full-sibships, and natural populations of *H. symbiolongicarpus.* Inbred strains such as the *G* line developed by MOKADY and BUSS (1996) may nevertheless prove useful in clarifying the details of the transmission genetics and matching criteria that govern allorecognition specificity in Hydractinia and other colonial invertebrates.

We are grateful to J. GILLESPIE, C. LANGLEY, P. MARKO, R. TOONEN, and anonymous reviewers for their helpful comments. Grants from the National Science Foundation to R.K.G. and D.R.L. supported this research.

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Communicating editor: S. L. ALLEN