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THE EVOLUTION OF ALLORECOGNITION SPECIFICITY IN CLONAL INVERTEBRATES

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ABSTRACT

Sessile, clonal invertebrates frequently encounter conspecifics as they grow over hard substrata and compete for space. Allorecognition systems mediate the nature and outcomes of these encounters by controlling somatic compatibility (fusion versus nonfusion) and agonistic behavior (aggression versus nonaggression). In general, clonemates (and sometimes close relatives) can fuse somatically, whereas more distant relatives are incompatible. Many anthozoan and hydrozoan cnidarians behave passively when in contact with clonemates and close kin, but fight aggressively when contacting more distant relatives. This high degree of allorecognition specificity, when considered together with the few available data on the formal genetics of allorecognition, suggests that levels of polymorphism at allorecognition loci (i.e., allotypic variation) exceed by perhaps an order of magnitude the levels typical of other polymorphic loci. In this paper, I evaluate the strengths and weaknesses of the selectionist and nonselectionist theories that have been proposed to account for the evolution and persistence of allotypic polymorphism. It remains difficult to accept or reject any of these hypotheses in the absence of detailed empirical information concerning levels and patterns of allelic variation at allorecognition loci. Nevertheless, mathematical considerations and the available data together suggest that frequency-dependent or spatially variable selection are the strongest candidates for the maintenance of allotypic variation. Although the pleiotropic effects of allorecognition loci (e.g., the regulation of gametic compatibility or pathogenic defense) could maintain allotypic variation, pleiotropy by itself does not account for the widespread evolution of aggressive behavior and somatic compatibility conditioned by allotypic similarity. It is theoretically possible that frequency-dependent selection acting at the level of the individual maintains allotypic polymorphism by restricting somatic fusion; it is less clear how individual selection maintains allotypic variation through the regulation of agonistic behavior.

> *"Any search for the biological significance of histocompatibility antigens must start with the firm postulate that their biological significance lies in their diversity and multiplicity and that means exist to ensure that such diversity is maintained."*
>
> W. F. Bodmer, *Nature*, 245: 359–361 (1973).

INTRODUCTION

ALLORECOGNITION comprises a series of events primed by contact between genetically dissimilar conspecific tissues and followed by a specific response to nonself that maintains the integrity of self. Precise allorecognition was once thought to be restricted to the mammalian immune system. Over the past twenty years, however, an increasing number of studies employing tissue grafting and behavioral assays show that many sessile, clonal invertebrates—including sponges, cnidarians, bryozoans, and ascidians—can discriminate conspecific self tissues from nonself tissues with great precision (Hildemann, 1979; Buss, 1982).

To confer a given level of specificity in self/nonself discrimination, there must exist a comparable amount of variation in cell-surface antigenic determinants involved in allorecognition. If it is assumed that such allotypic specificity is genetically based, then as allorecognition precision increases in a population, levels of genetic polymorphism at loci controlling specificity must also increase. This requirement, in turn, suggests that levels of polymorphism at loci that control allorecognition specificity may greatly exceed levels characteristic of other loci. For example, polymorphic loci encoding soluble and structural proteins typically carry fewer than a dozen allelic variants (Lewontin, Ginzburg, and Tuljapurkar, 1978; Nevo, Beiles, and Ben-Shlomo, 1984). In contrast, a single population of the colonial ascidian *Botryllus schlosseri* may carry as many as 100 alleles at the locus controlling allorecognition specificity (Grosberg and Quinn, 1986; Grosberg, 1987).

The major questions I will analyse in this review are straightforward: How and why are such high levels of allotypic polymorphism maintained at loci that control allorecognition? These apparently simple questions continue to baffle immunologists studying allorecognition specificity in mammalian systems (Burnet, 1973; Klein, 1979, 1982; Buss and Green, 1985); after all, how could such a precise system of allorecognition persist over evolutionary time, given that conspecific tissue interactions occur rarely during the life cycles of aclonal (or unitary) organisms, such as mammals?

It may be considerably easier to understand the functional significance of allotypic variation in sedentary, clonal organisms because of their distinctive developmental, demographic, and ecological attributes. In contrast to aclonal organisms, in which a zygote gives rise to a single soma, clonal organisms grow by the iteration of somatic units and can propagate asexually (reviewed in Hughes and Cancino, 1985). Consequently, the genotype (or "genet," *sensu* Harper, 1977) of a clonal organism is composed of numerous potentially discrete somata ("ramets," *sensu* Harper, 1977), which can grow, reproduce, and die independently of one another. This iterated organization lifts the surface-to-volume and structural constraints that must inevitably restrict the size of aclonal organisms, hence the ultimate size of a clonal genet may be intrinsically unlimited (Jackson, 1977, 1979; Sebens, 1979; Hughes and Cancino, 1985). The potential for indeterminate growth and reproductive output in clonal organisms may also defer the onset of senescence (Medawar, 1952; Williams, 1957; Hamilton, 1966), and probably underlies the exceptionally long genet lifespans found in several clonal taxa (Connell, 1973; Hughes and Jackson, 1982, 1985; Hughes, 1984; Cook, 1985).

As first described by Bancroft (1903), the combination of potentially indeterminate growth of a soma over a substratum, asexual multiplication, and longevity provides numerous opportunities for tissue contacts between isogeneic (self) and allogeneic (nonself) individuals in sedentary clonal invertebrates. Furthermore, limited dispersal of the sexual and asexual propagules of many clonal invertebrates often leads to dense aggregations of conspecifics (reviewed in Jackson, 1986). Thus, among sedentary clonal taxa—including sponges, bryzoans, ascidians, as well as many cnidarians—the function of genetic variation at allorecognition loci can be examined in a context where the capacity to distinguish self from nonself may be of paramount importance both in maintaining the integrity of self, and in determining the nature and outcomes of competitive and aggressive encounters.

Although much recent attention has been devoted to documenting allorecognition polymorphism in populations of clonal invertebrates, little effort has been given to critically analysing the evolutionary forces responsible for maintaining allotypic variation. In this pa-

per, I will evaluate the strengths and weaknesses of five classes of evolutionary theory that claim to account for the maintenance of allotypic genetic variation. Three of the hypotheses invoke natural selection. Frequency-dependent selection requires that individuals carrying rare allotypic determinants have higher fitness than individuals carrying common markers (e.g., Bodmer, 1972; Burnet, 1973; Wakeland and Nadeau, 1980; Levin, 1986). The mechanisms by which frequency-dependent selection acts are largely unknown, but the possibilities range from pathogen resistance, through protection against intraspecific parasitism, to kin recognition. The second selectionist theory involves overdominance: individuals heterozygous at allorecognition loci are presumed to have higher fitnesses than homozygous individuals presumably because heterozygotes resist infection from a broader range of pathogens (Robertson, 1982).

Spatial or temporal variation in selection pressures, such that the fitness of a particular genotype changes in space and time, can also maintain genetic variation. Spatial variation may be an especially important selection force acting on sedentary organisms, because individuals are largely incapable of averaging the effects of selection by moving among patches (Gillespie, 1975; reviewed in Hedrick, 1986). For such models to be applicable to the problem of allotypic variation, however, it must be assumed that allorecognition loci have direct effects on fitness, and that fitness varies spatially or temporally. These are distinct possibilities, especially because there is some evidence that pathogen resistance in mammals is correlated with particular allotypic combinations, and pathogen distribution certainly varies spatially (Bodmer and Bodmer, 1978).

In contrast to hypotheses invoking selection, Reimann and Miller (1983) proposed that allotypic variation is selectively neutral; thus, levels of variation at allorecognition loci represent a balance between mutation rate and losses that are due to genetic drift. Finally, in what may be viewed as an extension of the neutralist view, Ohno (1969) and Ohno and Wallace (1983) proposed a synergistic model for the accumulation of variation whereby high levels of polymorphism promote further variation by intragenic recombination and repair.

Each of these hypotheses makes specific assumptions about the nature and importance of natural selection and yields distinct predictions about levels and patterns of allotypic variation. One of the major goals of this paper is to articulate precisely these assumptions and predictions so that the models can be rigorously tested. Once these premises and predictions are set out, the power to test the hypotheses rests on the quality of the available data. At this point, and into the foreseeable future, it seems unlikely (at least for most clonal taxa) that direct assessment of patterns and levels of allelic variation will be possible; no efficient techniques exist that are comparable to the electrophoretic and serological methods used to measure variation at other loci and in other taxa. Indirect methods must therefore be used to estimate these data. Despite the empirical problems posed by the conspicuous absence and ambiguity of many critical data, I have organized the data into a form that allows some of the premises and predictions of the five theories to be evaluated critically.

This review has been arranged into six sections. The first provides an operational definition of allorecognition. The second outlines the pertinent evolutionary theories, emphasizing the data needed to evaluate the theories. The third section presents the available data on the formal genetics of allorecognition and the results of allorecognition assays that are the foundation for quantifying the levels and patterns of allotypic variation. The fourth section analyses the problems of reckoning the levels and patterns of allotypic variation based on indirect assays, and it interprets the data in the light of these difficulties. With this foundation in place, the fifth part examines the theories in the context of the available data. The final section analyses some of the functional consequences of allorecognition in sedentary clonal invertebrates, and explores whether these consequences can account for the maintenance of allotypic variation and individuality.

ALLORECOGNITION DEFINED

Allorecognition, historecognition, histocompatibility, or somatic compatibility systems may be characterized operationally by the expression of incompatibility following tissue contacts between allogeneic (i.e., genetically distinct) conspecifics, and by compatibility fol-

lowing isogeneic (i.e., self) tissue contacts. An operational allorecognition system requires that there be (1) a set of cues that confers allotypic specificity, (2) a mechanism that recognizes allotypic differences, and (3) an effector mechanism that is set in motion by the recognition system and that will result in symptoms of either incompatibility or compatibility.

The expression of incompatibility is clearest when rejection or aggression, accompanied by cytoxicity and tissue damage, follows allogeneic tissue contacts. Compatibility, on the other hand, is most apparent when tissue fusion follows allogeneic or isogeneic interactions (Hildemann, 1979). The failure to deploy an effector mechanism cannot, by itself, be taken as evidence for the existence of an allorecognition system. The absence of a response (i.e., nonfusion or passive behavior) must be coupled with observations of fusion or aggression in order to draw such an inference.

An individual's combination of alleles at the loci (or locus) controlling allorecognition is known as its allotype (or haplotype). For the sake of simplicity, I use the term "allotype" throughout the paper. Operational definitions of allotype and haplotype may be difficult to formulate in the absence of formal genetical data and because not all differences at loci that control allorecognition may be expressed, or recognized, as nonself. For example, in *Botryllus schlosseri*, individuals may differ by one allele at the allorecognition locus and still fuse. A further complication is introduced by data implying that incompatibility may be a quantitative, as well as a qualitative, response. Hildemann (1979), Scofield and Nagashima (1983), and Koyama and Watanabe (1983) have all suggested that variation in the intensity of allograft rejection is a function of the degree of genetic disparity between graft and host. These observations are reminiscent of vertebrate histocompatibility systems where a few loci have a major impact on allorecognition, and numerous other loci (termed minor histocompatibility loci) have smaller, but additively important, effects on allorecognition (Cohen and Collins, 1979).

THE THEORIES

The amount of variation at any locus is regulated by random and deterministic forces that act separately, or in concert, to augment, decrease, or stabilize existing variation. Genetic variation is added to gene pools by mutation and by gene flow from populations with different alleles. Random genetic drift can lead to either the loss or the fixation of a variant, the probabilities of each consequence being dependent largely on the initial frequency of the variant. Even a highly beneficial allele can be lost by drift, especially when it is rare and the population is small. Finally, natural selection, although incapable of increasing the number of variants in a population, can either increase or decrease the likelihood that a new variant will persist.

Each of the five theories under discussion here incorporates the forces of mutation, drift, and selection into its structure, but with different relative importances. None ignores the role of mutation and immigration. The frequency-dependent, heterotic, and spatial variation models, however, assign a major role to natural selection in maintaining variation, whereas the neutral and synergistic models weight the roles of mutation, recombination and drift more heavily than that of selection. All five models can account for the maintenance of allelic variation, but they differ in their assumptions about the way the variation is maintained, in their predictions about the amount of variation that can be maintained, and in the spatial and frequency distributions of this variation.

Frequency-Dependent Selection

The search for a mechanism capable of maintaining extensive polymorphism dates back to the discovery of large amounts of allelic variation at loci controlling compatibility between pollen and style in flowering plants (Emerson, 1938, 1939; Wright, 1939; de Nettancourt, 1977). Many angiosperms cannot self-fertilize, and compatibility reactions between pollen and maternal stylar tissues are genetically controlled (Mulcahy and Mulcahy, 1983). In gametophytic compatibility systems, pollen-tube growth and fertilization can proceed only if the haploid pollen grain and diploid stylar tissue do not share a compatibility allele. Pollen grains which share an allele with the recipient style either do not germinate, or do not grow successfully through the style. Given these genetic rules, any pollen grain carrying an allele that is rare relative to the frequencies

of other compatibility alleles in a population should be able to fertilize a disproportionately large number of ovules. All else being equal, the fitness of a compatibility allele under these conditions is inversely proportional to its frequency: rare alleles should increase in frequency simply because they are rare (Wright, 1939; Fisher, 1961; Ewens, 1969; Nagylaki, 1975).

If rare alleles at allorecognition loci have a selective advantage analogous to that of rare alleles in angiosperm gametophytic self-incompatibility systems, then frequency-dependent selection may underlie the maintenance of allotypic polymorphism among clonal invertebrates. For example, the appearance of a new allorecognition allele confers a novel identity upon the individual that possesses the new mutant. If individuality has a selective advantage, then rare alleles may have a large selective advantage after they enter the population through mutation or immigration. I will postpone discussion of the mechanisms that might favor individuality until the last part of this review; for the time being, I will ask two questions: (1) how many alleles can be maintained in a population under the assumptions of frequency-dependent selection acting on allorecognition loci, and (2) what should allelic frequencies be at equilibrium?

The role of frequency-dependent selection in maintaining genetic polymorphism has been studied in a variety of selection regimes (Ayala and Campbell, 1974). It is a difficult problem to study analytically because, by definition, the fitness of an allele is not fixed (Ewens, 1969). The following models implicitly assume that allorecognition loci have no phenotypic effects other than that of dictating allotypic specificity. Consequently, allelic variants have selective advantages that depend solely on their frequencies. Ewens (1969) states that rare alleles have a "quasi-selective advantage" that is evident only when the allele is rare.

Under strict conditions of frequency-dependence (such as those in the plant gametophytic compatibility system described above), the fitness of a novel mutant is

$$S_i = 1 - p_i, \qquad (1)$$

where S_i is the fitness of the ith allele, and p_i is the frequency of the ith allele. Because a newly arisen allele has a frequency very near to 0, it has an initial selective advantage, relative to other alleles, very near to 1. In terms of fitness relative to the population, the selection coefficient favoring a newly arisen allele is

$$SC_i = 1 - \omega \qquad (2)$$

where SC_i is the selection coefficient favoring allele i, and ω is the mean fitness of the population at genetic equilibrium.

At genetic equilibrium, under frequency-dependent selection, such as that potentially operating on compatibility systems, alleles should be equally common (Fisher, 1958; Ewens, 1964; Wright, 1969; Nagylaki, 1975). Hartl (1983) defines the mean fitness of a population (with respect to its allorecognition alleles under frequency-dependent selection) as

$$\omega = [(n - 2)(n - 1)]/n^2, \qquad (3)$$

where n is the number of equally frequent alleles. Substituting equation (3) into equation (2) gives

$$SC_i = (3n - 2)/n^2. \qquad (4)$$

This equation implies that selection coefficients favoring new alleles depend upon the number of alleles already in the population (Fig. 1); as the number of alleles at equilib-

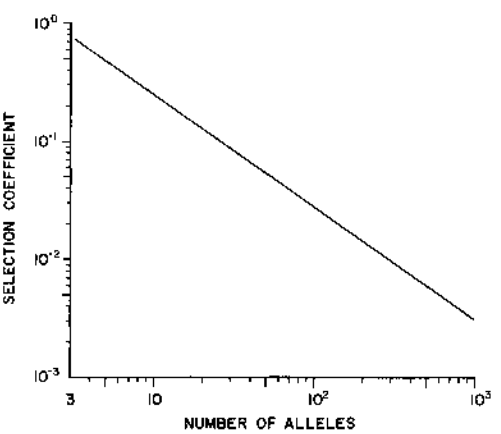

FIG. 1. THE SELECTION COEFFICIENT (SC_i) FAVORING A NEWLY ARISEN ALLORECOGNITION ALLELE

SC_i varies according to the number of extant alleles under frequency-dependent selection at a self-incompatibility locus.

rium increases so, too, will the mean fitness of the population. It therefore becomes progressively more difficult to add a new allele to a population because the relative selective advantage of a new allele decreases with increasing n.

Given that a newly arisen allele has a selective advantage that is inversely proportional to the number of alleles already in the population, how rare must a new allele be in order to increase in frequency? Most population geneticists have assumed that an allele rarer than the average allelic frequency (which, at equilibrium, is $1/n$) must increase until it reaches the frequency $1/n$. Nagylaki (1975) however, analysed the lower limit for selection acting to increase the frequency of an allele, and found that the $1/n$ rule is not robust. For a system of n alleles, if we neglect mutation and genetic drift, the completely symmetric equilibrium (i.e., all alleles are equally frequent) is locally stable: any allelic frequency less than q will increase, where q is defined as

$$q = 1 + a - [(1 + a^2)]^{-2}, \qquad (5)$$

and where $a = 1/[2(n - 1)]$. For all n, q must be greater than $\frac{1}{2}n$; however, if $n \gg 1$, $q \cong \frac{1}{2}n$. Equation (5) suggests that in small populations with large numbers of alleles (relative to the effective population size), a newly arising allele may not be sufficiently rare to have an initial selective advantage. This conclusion is supported by Wright's (1939) calculation of the selection index, R:

$$R = (n - 3)/(n - 1). \qquad (6)$$

R is formally equivalent to the mean fitness of a population, θ, and $1 - R$ can be viewed as the selection coefficient in favor of a new allele.

Not only does the selective advantage of a novel allele decrease as the number of extant alleles in a population increases, but the asymptotic rate of approach to equilibrium is $1/n$. The rate of approach to equilibrium therefore decreases with increasing n (Nagylaki, 1975), and it seems unlikely that most populations persist long enough to be in a state of true genetic equilibrium (Ewens, 1964).

Finally, the probability that an allele, once arisen, will be lost from a population is a consequence of effective population size, N_e (a function of the number of individuals contributing gametes to the next generation and their relative gametic contributions), the frequency of the allele, and the selection coefficient favoring the allele (Kimura and Crow, 1964). At genetic equilibrium under frequency-dependent selection there is no selection, and population size alone determines the rate at which an allele will be fixed or lost (Kimura and Crow, 1964; Wright, 1969). Thus, the equilibrium conditions are like those under the neutral-allele model (see p. 386-387 below): the number of alleles maintained will be a function of (1) population size and (2) mutation rate [see equation (9)]. If all alleles are equally common at genetic equilibrium (as would be the case under frequency-dependent selection acting on a compatibility system), Kimura and Crow's n is formally equivalent to the actual number of alleles at equilibrium under a frequency-dependent selection regime such as that of gametophytic incompatibility in angiosperms.

Unfortunately, allotypic variants are neutral only when equally frequent, and any perturbation from this equilibrium gives rarer alleles a selective advantage. This violates the fundamental assumptions of neutral allele models, thereby making exact predictions of the number of allorecognition alleles at equilibrium analytically intractable.

How, then, can one estimate the number of alleles that can be maintained in a population under frequency-dependent selection? The impetus to answering this question was provided by Emerson's (1938, 1939) observations that in a population of about 500 individuals of the endemic evening primrose *Oenothera organensis*, there were at least 45 alleles at the locus controlling pollen-style compatibility. This extraordinary allelic diversity led to a number of crucial, but rather complex, theoretical papers (Wright, 1939, 1960, 1964; Fisher, 1958; Moran, 1962; Crosby, 1966; Ewens 1964, 1969).

Novel alleles can appear only by immigration or mutation. Wright (1939) therefore approached the *Oenothera* problem by calculating the mutation rate necessary to maintain a given number of alleles in a given population size, with single-locus frequency-dependent selection. He assumed genetic equilibrium and an infinite number of possible alleles. Details of the model can be found in Wright (1939);

Fig. 2 summarizes graphically the predictions of the model, based on the relationships among effective population size, mutation rate, and number of alleles at equilibrium.

To appreciate its relevance to the problem of maintaining single-locus allotypic variation, it is worth examining Fig. 2 in terms of the maintenance of variation in *Oenothera*. A mutation rate on the order of 10^{-3} per locus per generation would be necessary to maintain 40 or so alleles, assuming that Emerson's estimate of a population size of 500 individuals is correct. If it is assumed that mutation rate has a more conventional value of 10^{-6} per locus per generation, then there are several other factors that could influence the value of n. The most obvious is that Emerson grossly underestimated population size. In fact, Ritter (cited in Levin, Ritter, and Ellstrand, 1979) estimated that the actual panmictic population of *Oenothera organensis* consisted of 5000 individuals, rather than the 500 estimated by Emerson. However, N_e would have to be greater than 10^4 in order to maintain 45 alleles with a mutation rate of 10^{-6}. Therefore, if the population size estimates are roughly correct, and the population is panmictic, it appears that frequency-dependent selection alone cannot account for the variation in *Oenothera organensis*.

An alternative, and complementary, explanation assumes that the population is subdivided into a number of demes among which gene flow is restricted or absent. Under this scenario, the rate of loss of alleles that is due to drift might be higher than in a panmictic population, but because the selective advantage of a mutant allele is inversely proportional to the number of extant alleles, any new mutant would have a greater selective advantage in a smaller population. Wright (1960, 1964) realized this possibility, and proposed that the population of *Oenothera* may have been subdivided into many small groups with restricted gene flow among them. For example, an isolated subpopulation of 50 individuals would maintain 5 alleles at a mutation rate of 10^{-6}. If this population of 50 were, in turn, subdivided into 10 isolated groups of five each, then about three alleles could be maintained per group, with about 30 alleles for the groups summed together. In general, the greater the number of subgroups, the smaller their size, and the greater the restriction of gene flow among subgroups, the greater the amount of allelic variation that can be maintained in the species as a whole. This subdivision hypothesis, although theoretically plausible and appealing, is not supported by the genetic structure of the *Oenothera organensis* population: Fisher (1961) showed that the spatial distribution of self-incompatibility alleles was homogeneous, and Levin, Ritter, and Ellstrand (1979) showed that what little allozyme variation there was in the population was distributed homogeneously among sites.

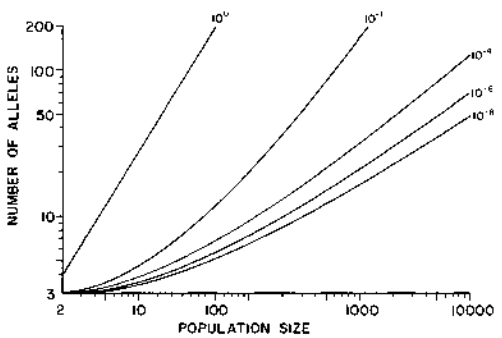

FIG. 2. THE NUMBER OF ALLORECOGNITION ALLELES MAINTAINED IN A POPULATION AT FIVE MUTATION RATES

The number maintained is a function of population size and of frequency-dependent selection for self-incompatibility alleles; it is assumed that there is an infinite number of possible alleles. After Wright (1939).

Wright (1960) also proposed that the problem of maintaining self-incompatibility alleles in *Oenothera* could be resolved if the population were not in genetic equilibrium, and represented a relict of a formerly much larger population. The amount of genetic variation persisting in a relict population would depend upon (1) selection at the loci under consideration; (2) time since isolation of the population; (3) number of subgroups within the isolated population; (4) effective population size; and (5) mutation rates (Ewens, 1964; Wright, 1969; Lewontin, 1974). Such detailed historical and genetical information is presently unavailable for *Oenothera* in particular, and for most other species, in general.

As a final complication (or solution), Ewens (1964, 1969) argued that stationary models of the sort used by Fisher and Wright are inap-

propriate for predicting the number of alleles under frequency-dependent selection because the fitness of an allele is not deterministic. Rather, the fitness of an allele is dynamic, and depends upon its frequency. This suggests that a fundamental assumption of the conventional diffusion approach to this problem is violated: transition probabilities are not fixed, or even close to being fixed, except when n is very large (Ewens, 1964). Similarly, Kimura and Crow's neutral allele models are inappropriate because they assume that alleles are selectively neutral regardless of their frequencies. Indeed, Ewens concluded that the residence time of an allele would be increased over that predicted by the models of Fisher, Wright, Kimura and Crow because the selective advantage of a rare allele increases as its probability of extinction increases. This, coupled with Nagylaki's (1975) calculation that the rate of approach to equilibrium decreases with increasing n, produces a quasi-equilibrium that effectively increases levels of allelic variation without necessarily invoking unrealistically high mutation rates or population sizes.

Despite the difficulty of finding a single solution to the *Oenothera* problem, there are several valuable lessons to be learned. The first is that frequency-dependent selection has the power to maintain large amounts of allelic variation at allorecognition loci. This power depends explicitly on both mutation rates (along with immigration rates) and effective population size. In turn, effective population size will depend upon the number of breeding individuals and patterns of subdivision among groups of breeding individuals. These conditions point to the second valuable lesson: mutation rates, gene flow, allelic frequencies, and the genetic architecture of populations must be carefully examined to assess the role played by frequency-dependent selection. Finally, I stress that all of the models presented in this section refer to the maintenance of single-locus polymorphisms; the problem of maintaining multilocus polymorphisms under frequency-dependent selection has yet to be examined theoretically (Bodmer and Bodmer, 1978).

Variable Selection

On theoretical grounds (reviewed in Hedrick, 1986), temporally varying selection, by itself, should be a relatively weak force in the maintenance of extensive polymorphism. In contrast, spatially varying selection is potentially capable of maintaining substantial amounts of allelic variation. Most theoretical studies of the effects of spatially varying selection on the maintenance of polymorphism specify a temporally constant, spatially variable selective regime, and then allow genotypes, whose fitnesses vary specifically by patch or niche, to recruit into this structured environment (reviewed in Felsenstein, 1976; Hedrick, 1986). Levene (1953) first showed that a two-allele polymorphism could be maintained in a two-patch environment, but only if the two homozygotes have harmonic mean fitnesses of less than one. The most favorable conditions for protected polymorphism occur when fitness differentials between patches are large and migration between patches is low. However, even with strong selection and limited migration (or habitat selection), most models require the restrictive condition that relative niche frequencies ("sizes," *sensu* Maynard Smith and Hoekstra, 1980) be nearly equal in order to maintain stable polymorphisms (Hedrick, Ginevan, and Ewing, 1976; Maynard Smith and Hoekstra, 1980; Hedrick, 1986).

Gillespie (1978) proposed a general model of spatially varying selection to account for the maintenance of isozyme variation. The assumptions of this model are shown in detail in Table 1. Specifically, patches vary randomly, and enzymatic activity for different alleles is additive; consequently, enzyme activity can be ranked on a stochastic additive scale (SAS). Additionally, the function, $\phi(x)$, relating the enzymatic activity of a genotype, x, to its fitness is concave downward and increases monotonically to some asymptote (i.e., a concave fitness function—CFF). It is also necessary to assume that the fitness of a heterozygote in a given patch or niche lies between the fitnesses of its associated homozygotes, and exceeds the mean of the two homozygotes (i.e., there is an arithmetic mean heterozygote advantage). With these assumptions, stable polymorphism requires that the two homozygotes differ in their niche-specific fitnesses. If this is the case, then even relatively small niche-specific selective advantages (on the order of 0.01) lead to a stable polymorphism over a broad range of relative niche frequencies (Maynard Smith and Hoekstra, 1980).

TABLE 1

Assumptions of the SAS (stochastic additive scale)-CFF (concave fitness function) model for the maintenance of polymorphism by spatially varying selection

After Maynard Smith and Hoekstra (1980).

	Genotype		
	A_1A_1	A_1A_2	A_2A_2
Enzyme Activity	x_1[a]	$(x_1 + x_2)/2$	x_2
Fitness	$\phi(x_1)$[b]	$\phi[(x_1 + x_2)/2]$	$\phi(x_2)$
Niche-specific fitness	1	$1 - hs$[c]	$1 - s$

[a] x is the enzymatic activity of a genotype.

[b] ϕ is the function relating enzymatic activity to fitness.

[c] h specifies the heterozygotic fitness effect of an allele compared to the allele's homozygotic effect; s is the selection coefficient.

Analytical extension of two-allele models to the case of n alleles is mathematically quite complex. Strobeck's (1979) single-locus haploid model suggests that the number of selectively different niches, m, must be greater than or equal to the number of alleles maintained. His model did not specify the stability conditions for multiallelic polymorphism. Gillespie (1977) modified the SAS-CFF isozyme model for the case of multiallelic polymorphism and concluded that, "The condition for polymorphism for n alleles requires, roughly, that the mean difference in activity between neighboring homozygous genotypes is less than twice the variance in [enzymatic] activity divided by the square of the number of alleles" (Gillespie, 1977:90). This result, however, is subject to the same restrictions as the two-allele model described above (Maynard Smith and Hoekstra, 1980). Although there is empirical evidence that the assumptions are met at some allozyme loci (reviewed in Hedrick, 1986), whether the necessary fitness scaling and selective assumptions are met at other loci, including those regulating allorecognition, remains unexplored.

These theoretical analyses together indicate that several conditions must be met in order that spatially varying selection maintain substantial levels of genetic polymorphism. First, there must be some form of marginal overdominance (*sensu* Wallace, 1968) such as that assumed by Gillespie (1977, 1978). Second, it is empirically necessary to identify the selective pressures that affect the relevant loci, and to show that these pressures vary spatially. If spatially varying selection does maintain allotypic polymorphism, then (1) the number of selection regimes should approximate the number of alleles, (2) allotype-specific fitness should be related to specific patch types, and (3) allotype frequencies should vary according to the frequencies of patch types that favor specific allotypes.

Heterosis

Heterosis, or overdominance, refers to allelic combinations in which a heterozygote has greater fitness than either of its homozygotes (i.e., the fitness of genotype AB > AA and BB). In theory, heterosis can lead to the maintenance of a balanced polymorphism; in practice, the evidence for heterozygote advantage being important in nature is limited to a few well-cited examples (reviewed in Clarke, 1979).

The history of models accounting for the maintenance of allotypic polymorphism through heterozygote advantage can be traced to the statistical association between disease susceptibility and specific allotype in humans (reviewed in Bodmer and Bodmer, 1978). If disease resistance is conferred by a particular allorecognition antigen, then it is intuitively appealing to argue that a heterozygote has a broader window of resistance than a homozygote. For example, Robertson (1982: 629) states, "Thus, an animal heterozygous for MHC [major histocompatibility complex] antigens may respond efficiently to a wider range of pathogens than a homozygote, and polymorphism may be maintained by heterozygous advantage." There is some evidence to support the premise of the argument, but the conclusion is too vague to evaluate: the pertinent question is, How much polymorphism can heterosis maintain?

The justification for invoking heterosis for the maintenance of multiple-allele polymorphisms rests on the intuitive premise that the existence of a balanced polymorphism of two alleles can be extended to multi-allelic systems. Gillespie (1977), as well as Lewontin, Ginzburg, and Tuljapurkar (1978), have modeled the limiting conditions for the maintenance of a stable polymorphism by means of heterosis. Specifically, Lewontin, Ginzburg, and Tuljapurkar (1978) showed that heterozygote advantage can maintain a stable three-allele polymorphism only under very stringent conditions.

For heterosis to maintain still more allelic variation would require that (1) all heterozygotes are more fit than all homozygotes and (2) all heterozygotes have nearly equal fitnesses. In the unlikely event that these requirements were met in practice, between six and ten allelic variants, at approximately equal frequencies, appear to be the maximum amount of polymorphism that heterosis can maintain in a population.

These theoretical analyses suggest that the role of heterosis alone in the maintenance of single-locus allotypic variation may be small. If allotypic variation were distributed among loci in such a way that allotypic specificity was determined by many gene-products rather than the products of a single-locus, would greater specificity be possible? At first sight, the answer seems to be yes. For example, the number of unique genotypes per locus is:

$$[n(n + 1)]/2, \tag{7}$$

where n is the number of alleles at the locus. If loci are unlinked, then the number of unique genotypes at L loci is:

$$([n(n + 1)]/2)^L. \tag{8}$$

Thus, fewer alleles per locus would be needed to maintain a comparable number of allotypes if allotypic specificity were conferred by the gene-products of independent loci. This consideration appears to reduce the burden on heterozygote advantage. Unfortunately, the loci cannot be considered to be independent, even if located on separate chromosomes, for it is their combined effects that are selectively important. Therefore, epistasis raises the same problems as the maintenance of a single-locus polymorphism by means of heterosis (Gillespie, pers. commmun.).

The model of frequency-dependence outlined previously predicts that all alleles should be equally frequent at genetic equilibrium. At equilibrium with many alleles, heterosis should also result in roughly equivalent allelic frequencies (Lewontin, Ginzburg, and Tuljapurkar, 1978). These models differ in that frequency-dependence will lead to genotypic frequencies that agree with Hardy-Weinberg equilibria, whereas heterosis will produce an excess of heterozygotes over that expected at Hardy-Weinberg equilibrium.

There are, as usual, practical difficulties to using genotypic frequencies to distinguish among competing models. First, processes other than heterosis can lead to deviations from Hardy-Weinberg expectations. Second, in a highly polymorphic system, most genotypes will be rare, hence precisely estimating their frequencies would require an enormous sample of genotypes. Consequently, detecting deviations would be statistically unlikely.

When the theoretical arguments and empirical data are considered together, there is little evidence that heterosis alone can maintain more than a few allotypes in a population. Nonetheless, as Neigel (1988) recently suggested, heterosis may work in concert with frequency-dependent selection to augment allotypic variation.

Selective Neutrality

The neutral allele theory was first proposed by Kimura (1968) and King and Jukes (1969) partly to explain the persistence of large amounts of allozyme variation. Neutral models suggest that allelic variation at some polymorphic loci is selectively neutral; that is, as far as natural selection is concerned, different alleles produce phenotypes of comparable fitness. In the context of allorecognition systems, Reimann and Miller (1983) were the first to propose that allotypic variation has little, or no, adaptive significance. In their view, levels of allotypic variation represent a balance between the introduction of novel variation and the loss of variation that is due to random effects.

As I discussed in the previous subsection on frequency-dependent selection, the number of alleles that can be maintained in the absence of selection depends upon mutation and immigration rates, as well as upon effective population size. Kimura and Crow (1964) showed that the effective number of selectively neutral alleles that can be maintained at equilibrium is:

$$n_e = 4N_e\mu + 1, \tag{9}$$

where n_e is the effective number of alleles, N_e is the effective population size, and μ = mutation rate to neutral alleles. Fig. 3 shows that for neutral models to account for allelic variation in excess of 10 alleles per locus, some combination of large population size or high mutation rate is necessary. Thus, quantification of effective population size and mutation rates,

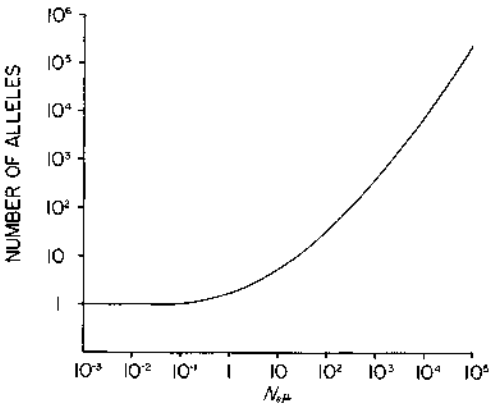

FIG. 3. THE EFFECTIVE NUMBER OF SELECTIVELY NEUTRAL ALLELES AT GENETIC EQUILIBRIUM

The number is a function of the product of effective population size (N_e) and of mutation rate to new alleles (μ). After Lewontin (1974) and Hartl (1983).

as well as allelic and genotypic frequencies (see Hedrick, Thomson, and Klitz, 1982) are needed before the importance of neutrality can be weighed against selective theories.

Synergistic Models and Intragenic Recombination

Mutation rate plays a critical but relatively minor role in maintaining variation under frequency-dependent selection. In contrast, mutation rates play a major role in neutral models; indeed, usual estimates of mutation rates on the order of 10^{-5} to 10^{-6} per locus per generation severely limit the power of neutral models to explain extensive polymorphism.

Molecular geneticists have long known that recombination between sites within a gene can lead to the production of novel allelic variants (Bodmer and Darlington, 1969; Ohno, 1969). Intragenic recombination, or gene conversion, leads to what Futuyma (1979) has termed "pseudomutational" events. Ohno (1969) proposed that polymorphism, once established, could beget further polymorphism by intragenic recombination, potentially leading to mutation rates considerably higher than estimates based on the appearance of deleterious mutants.

The essence of Ohno's (1969) mechanistic model is that repair of inevitably mismatched DNA base-pairs after recombination leads to "mutant" products. Imagine two homologous chromosomes, each carrying a different allelic variant at a particular locus. These allelic variants must differ by at least two nonconsecutive base substitutions. If crossing over occurs between two non-sister chromatids peripheral to the locus in question, then upon repairing after crossing over, there will be some mismatching of nucleotide base pairs. For perfect rematching, one base of each mismatched pair must be repaired: if this repair process occurs at random on either the transcribing or complementary DNA strand, then it can yield as many as two new allelic variants. The more alleles there are, and the more nonconsecutive base substitutions, the more new variants can appear by intragenic recombination. In this way, the rate at which new alleles can be generated at a locus may exceed by several orders of magnitude the rate suggested by spontaneous mutation events (Watt, 1972; Golding and Strobeck, 1983).

To assess the general impact of intragenic recombinational events on estimates of mutation rates, a broad sample of nucleotide sequences of allotypic variants would be required. These data are not yet available even for well-defined loci; consequently, the mechanisms producing novel variants and the rates at which novel variants are produced remain matters of speculation.

GENETICS OF ALLORECOGNITION, COMPATIBILITY FREQUENCIES, AND THE SPATIAL DISTRIBUTION OF ALLOTYPES

To distinguish empirically among the theories, it is necessary to have some estimate of (1) the number of loci controlling allorecognition, (2) the number and frequencies of alleles at each locus, and (3) the spatial distributions of allotypes within populations for a variety of clonal invertebrates. Ideally, data on levels of gene flow, effective population size, and mutation rates would also be available.

Genetics of Allorecognition

The foundation of such a data base is an understanding of the formal genetics of allorecognition. Without this information, it is nearly impossible to specify how variation is distributed within and among loci, and therefore

to determine levels of polymorphism. There are two related formal genetic questions that must be addressed before levels of allelic variation and allotypic frequencies can be determined. The first question focuses on the genetic rules of compatibility: Must all allotypic determinants be shared if two individuals are to be compatible, or is it sufficient for some fraction of allotypic determinants to be shared? An answer to this question is critical because most estimates of allotypic frequencies are based on the results of grafting or behavioral assays, rather than direct determination of allotypes. If only partial matching is required for compatibility to occur, then compatibility frequencies reported from allorecognition assays may underestimate allotypic diversity. The second question is, How many loci are involved in allorecognition? Allotypic specificity could result from the combination of effects of many loci, each with a few alleles, or of a few loci, each with many alleles (Curtis, Kerr, and Knowlton, 1982).

The most powerful method for answering these questions is formal genetic analysis through breeding studies. The technical difficulties of breeding experimental organisms have precluded, for the most part, direct genetic analysis. Consequently, precise estimates of the number of loci involved in allorecognition, and the genetic rules of compatibility, are virtually unknown. In lieu of genetic analysis based on defined matings, two indirect methods have been used to analyse the genetic rules of compatibility. The first method employs transitivity tests, in which compatibility relationships among groups of three individuals are examined. The second method compares genotypic assignments based on allorecognition assays versus some other independent marker of genetic identity, usually isozyme profiles.

Breeding Studies

The first steps toward a formal genetic analysis of allorecognition were taken by Bancroft (1903). His studies of the colonial ascidian *Botryllus schlosseri* showed that somatic fusion between unrelated colonies was unusual; however, allografts between parents and their progeny always fused, and grafts among siblings often fused. Subsequent breeding studies led to the first and only well-supported genetic model for allorecognition in any invertebrate (Oka and Watanabe, 1957, 1960; Sabbadin, 1962; Scofield, Schlumpberger, West, and Weissman, 1982). These studies consistently show that allotypes are primarily determined by alleles at a single, highly polymorphic, mendelian locus. In order for rejection to occur, two individuals must have no common alleles at this locus (e.g., if alleles are designated sequentially as A, B, C, . . ., then juxtaposing an AB and CD colony results in nonfusion). If two individuals share one allele (e.g., AB and BC) or both (e.g., AB and AB) alleles, then they can fuse.

Koyama and Watanabe's (1984) preliminary analysis of the genetics of compatibility between stolons in the social ascidians *Perophora japonica* and *P. sagamiensis* showed that F_1's did not fuse with either parent, but some F_1's fused with each other. These data suggest either that allorecognition is controlled by several loci, or that both alleles must be shared if a single locus controls allorecognition.

There are just a few glimpses into the formal genetics of sponge allorecognition. Van de Vyver (1970) and Van de Vyver and Willenz (1975) found that sexually produced sibling larvae of the sponge *Ephydatia fluviatilis* fused and gave rise to a single, chimeric sponge whereas mixtures of nonsibling larvae did not fuse. Moreover, asexually produced gemmules from each of two parental strains fused with sexual progeny from a mating between these parents, but did not fuse with progeny from matings between other parents. Van de Vyver (1970) reported a similar pattern in *Crambe crambe*: 75 per cent of full-sibling larvae were mutually fusible, and all F_1's fused with their mother.

Hauenschild (1954, 1956) investigated the formal genetics of allorecognition in the athecate hydroid *Hydractinia echinata*, and drew three tentative conclusions: (1) allorecognition is genetically controlled; (2) colonies derived from sibling planulae are generally fusible, whereas nonsibling colonies, upon contact, usually (but not always) produce hyperplastic stolons; and (3) all F_1 progeny are fusible with both of their parents. In contrast to Hauenschild's (1956) findings, Ivker (1972) found that progeny, in general, did not fuse with their parents, although a hyperplastic rejection response was not always produced in parent-progeny combinations. Buss (pers. commun.) reported that nearly all F_1's fuse with both parents, as well as with their siblings and half-

siblings; fusions are rarely found between unrelated individuals. Clearly, the genetics of histocompatibility in *Hydractinia* are far from being understood; however, closely related individuals may have sufficiently similar (if not identical) allotypic determinants such that allogeneic interactions produce no hyperplastic response. The observation that F_1's can fuse with both parents, however, suggests that complete allotypic matching is not a requirement for compatibility in *Hydractinia*.

Transitivity Analyses

Allorecognition studies that examine transitivity relationships among individuals provide further insights into the genetic rules of compatibility. If numerous three-way combinations of individuals yield transitive results (i.e., A is compatible with B, B is compatible with C, and C is compatible with A), then complete allotypic matching seems necessary for compatibility. However, if individual A fuses with B and C, but B does not fuse with C, then only partial allotypic matching is required for fusion.

Several allografting studies on sponges show complete transitivity, hence are consistent with the hypothesis that complete allotypic matching is required for compatibility (Neigel and Avise, 1983a,b, 1985; Neigel and Schmahl, 1984; Wulff, 1986). In contrast to these results, compatibility relationships are not transitive in ascidians (Mukai, 1967; Fuke and Numakunai, 1982; Fuke and Nakamura, 1985). On theoretical grounds, a large number of transitivity tests would be necessary to distinguish between the partial- and full-matching models of compatibility (Grosberg, Rice, and Palumbi, 1985; Wulff, 1986). Moreover, transitivity tests based on field samples of unknown genetic identity are potentially misleading without prior independent determination of the distance over which clonal fragments disperse. Consider the following three scenarios:

1. Assume that (a) dispersal of asexual propagules is limited, (b) dispersal of sexual propagules is extensive, and (c) grafting assays are performed only between individuals separated by distances greater than the range of movement of asexual propagules. Under these conditions, transitivity tests are likely to be based on allografts and therefore to be reliable tests of rules of matching (e.g., Neigel and Schmahl, 1984).
2. Make the same assumptions as in #1, but perform grafts among individuals within the bounds of dispersal of asexual propagules. In this situation, transitivity may be a trivial result because it is a priori being tested among clonemates. [Even grafts between distant individuals may not escape this problem; the data of Lasker and Coffroth (1985) suggest that clonal fragments of a gorgonian may be dispersed up to 1 km apart. Jackson (1985, 1986) reviews patterns of dispersal of clonal fragments.]
3. Assume that dispersal of sexual propagules is limited. Under these conditions, transitivity assays are likely to include both clonemates and siblings. As close relatives are likely to share all, or some, of their allodeterminants (depending upon their relatedness, the genetics of allorecognition, and the level of polymorphism), transitive relationships may involve combinations of allogeneic but closely related individuals.

Biochemical Genetic Studies

Over the past several years, a number of biochemical genetic studies on corals and sponges have suggested that allogeneic individuals are histocompatible. Using polyacrylamide gel electrophoresis, Curtis, Kerr, and Knowlton (1982) analysed the plasmalemmal proteins of compatible pairs of the sponge *Ectyoplasia ferox*, and found that some fusible individuals could differ electrophoretically. Jokiel, Hildemann, and Bigger (1982) and Neigel and Avise (1985) criticized these results on the grounds that electrophoretic differences between fusible sponges may have arisen because of sample contamination or ambiguities in the scoring of gels. Yet, recent studies of allozyme variation in another sponge, *Niphates erecta*, show that 18 per cent of fusible grafted pairs are electrophoretically distinguishable at three polymorphic loci (Neigel and Avise, 1985). Because only three loci could be reliably scored, the observed disparity between the allozyme and allorecognition assays must be a minimum estimate of the actual disparity.

Heyward and Stoddart (1985) simultaneously examined histocompatibility and electrophoretic variation in two species of the coral

Montipora. Of 40 fusible pairs of *M. dilatata*, 18 involved fusions between electrophoretically distinguishable pairs. In the case of *M. verrucosa*, 3 of 7 fusions involved electrophoretically distinct pairs. In another coral, *Pavona cactus*, electrophoretic methods resolved genotypes more precisely than grafting techniques (Willis and Ayre, 1985). Finally, in the corals *Porites cylindrica, P. nigrescens*, and *Seriatopora hystrix*, electrophoretically distinguishable individuals fuse; in all of these species, at least one fusible pair of grafts shared no alleles at at least one locus, implying that fusible individuals need not be siblings (Resing and Ayre, 1985).

Similar studies of clonal identity of the anemone *Actinia tenebrosa* using behavioral and electrophoretic methods are consistent with the results from coral grafting assays. Of 36 pairings between identical electrophoretic genotypes (at five polymophic loci), 7 elicited acrorhagial responses (i.e., responses involving the use of specialized tentacles) (Ayre, 1982). Of 53 pairings between different electrophoretic genotypes, only 20 elicited acrorhagial responses (Ayre, 1982). Thus, not all interactions between genotypes evoke an acrorhagial response. Insofar as acrorhagial responses are the result of the recognition of nonself, these results show that the genetic individual is not necessarily unique for allotypic determinants in *A. tenebrosa* (Bigger, 1980).

Hunter's (1985) analysis of clonal structure in a population of the coral *Porites compressa* contrasts with those cited in the previous paragraphs. Based on several putatively independent means of assaying clonal identity (including starch gel electrophoresis, colony morphology, grafting, and high performance liquid chromatography profiles of UV absorbing compounds), she concluded that grafting assays accurately reflected allotypic diversity in this species.

Compatibility Frequencies in Natural Populations

With the exception of colonial ascidians in the genus *Botryllus*, levels of allotypic variation have been inferred from allorecognition assays in the absence of a formal genetic foundation. In this section, I review the results of allorecognition assays that emphasize compatibility frequencies without specific attention to the dispersion of compatibility types.

Sponges

Hildemann and his coworkers were among the first to estimate population-wide frequencies of graft acceptance and rejection in an invertebrate. Their results demonstrated the potential for great variability in allotypic determinants. For example, Hildemann, Johnston, and Jokiel (1979) reported that in 200 grafts between physiologically discrete individuals of *Callyspongia diffusa*, there were no graft acceptances. In an extension of this initial study, there were no fusions in 480 grafts between physiologically discrete individuals from two "widely separated" reefs (Hildemann, Bigger, Johnston, and Jokiel, 1980: 32). From these data, they concluded that there was "extensive polymorphism of cell-surface histocompatibility molecules within this species" (Hildemann, Johnston, and Jokiel, 1979: 422).

Kaye and Ortiz (1981) documented fusion/rejection frequencies for the demosponges *Verongia* (= *Aplysina*) *longissima* and *V. cauliformis*. They attempted to test histocompatibility in all pairwise possibilities among 78 *V. longissima* and 13 *V. cauliformis*. They were unable to perform all grafts; hence, some of their results were inferred from transitivity assumptions. From these multiple-fusion tests, they inferred that there were 20 groups of mutually fusible individuals (= "strains" in their terminology) among 78 *V. longissima* individuals, and 1 "strain" among 13 *V. cauliformis*. Contrary to the results of Hildemann, Johnston, and Jokiel (1979) and Hildemann, Bigger, Johnston, and Jokiel (1980) on *Callyspongia diffusa*, a substantial number of putative allografts were fusible. This result may be taken to mean that allografts were, in fact, autografts, or else that allografts were fusible. Their data do not allow discrimination between these alternatives. In a similar study on *Hymeniacidon* sp., Curtis (1979) found that 32 per cent (16/50) of grafts between "nonneighboring" sponges were fusible.

Cnidarians

The most ambitious population-wide attempt to characterize allotypic polymorphism in cnidarians focuses on the hermatypic, scleractinian coral *Montipora verrucosa* (Hildemann et al., 1977; Hildemann, Jokiel, Bigger, and Johnston, 1980). Of 890 putative allografts,

none were compatible, whereas all autografts fused. Importantly, all grafts were made between individuals taken from reefs separated by nearly 50 km. They refer to the grafts as interpopulation grafts, implying that these geographically distinct populations are reproductively isolated.

In another extensive grafting experiment, Theodor (1966, 1970, 1976) found that of 2430 allografts of the gorgonian *Eunicella stricta*, 0.7 per cent were fusible. He did not specify the geographic extent of the collections used for the grafting experiments, or if *Eunicella* propagates asexually. I assume that the collections were made in a rather limited area and that there is little asexual propagation. The dispersal distance of *Eunicella* larvae is undescribed, although Theodor (1976) asserts that they are poor swimmers and likely to be spread by currents. If these two assumptions are accepted, it seems reasonable to presume that Theodor studied a panmictic population, and that the low frequencies of reported graft compatibilities, do, indeed, reflect substantial genetic polymorphism.

Anemones have been used frequently in studies of cnidarian allorecognition, in part because of their distinctive agonistic behavior in response to allogeneic contacts. This distinctive behavior, coupled with their extensive color and electrophoretic polymorphisms, make anemones particularly useful for studies of clonal population structure. The first studies of anemone allorecognition suggested that all clones were allotypically distinct. In 75 allogeneic contacts between individuals (putatively derived from different clones) of the clonally progating anemone *Anthopleura elegantissima*, acrorhagi were deployed in all trials; no acrorhagi were deployed when clonemates were juxtaposed (Francis 1973a). In another study of *A. elegantissima*, Lubbock (1980) found that 101 of 102 interclonal contacts elicited the acrorhagial response and subsequent nematocyst discharge. Similarly, Brace (1981) found an acrorhagial response in 97 per cent (79/82) of contests between nonclonemates of *Phymactis clematis*.

Although most anemone studies suggest that allogeneic contacts elicit aggressive responses, Bigger's (1980) study of *Anthopleura krebsi* showed that roughly half of putative allogeneic combinations produced no acrorhagial response.

Ascidians

Attempts at extensive grafting experiments in natural populations of colonial ascidians have focused on populations of *Botryllus primigenus* in Japan, and a population of *Botryllus schlosseri* from Woods Hole. Tanaka and Watanabe (1973) counted 11 fusions in 968 pairings (1.1%) of *B. primigenus* at Shimoda. In a later study, Mukai and Watanabe (1975a) tested 30 colonies (ten colonies from each of three stations about two or three kilometers apart) of *B. primigenus* in all possible pairwise combinations. One of these sites was the same as that studied several years earlier by Tanaka and Watanabe (1973). Over just a few years at this site, fusion frequencies had increased significantly to 5.8 per cent ($p < 0.05$, test of equality of percentages, Sokal and Rohlf, 1981). Karakashian and Milkman (1967) obtained one fusion in 22 random grafts (4.6%) of *B. schlosseri* from the Eel Pond at Woods Hole, Massachusetts (USA); in examining 1262 intercolonial borders in the field, they found 78 fusions (6.2%). Grosberg (1987) obtained similar results, finding a fusion rate of 4.4 per cent out of 500 pairs of randomly chosen colonies. Because it is generally impossible to distinguish chimerical from nonchimerical colonies in the field, some of the colonies used in these grafting studies undoubtedly were chimeras; thus, these data probably underestimate actual fusion frequencies.

Solitary ascidians do not propagate asexually, hence the physiological individual and the genetic individual are identical. With the ambiguous exception of tunic fusion in *Molgula complanata* (Schmidt, 1982), there are no known examples in which two solitary ascidians have become physiologically contiguous. Because natural fusions probably do not occur among solitary ascidians—hence the functional significance of allorecognition is obscure—little attention has been paid to their histocompatibility attributes. Nevertheless, in an elegant study of allogeneic interactions among coelomocytes of the solitary ascidian *Halocynthia roretzi*, Fuke (1980) demonstrated that self/nonself recognition occurs. The recognition of nonself is manifested by a distinctive, recipro-

cal, lytic reaction; most allogeneic cell cultures show extensive histolysis, whereas syngeneic cultures do not.

Using the "contact reaction" assay, Fuke and Numakunai (1982) characterized allotypic diversity in a local population of *Halocynthia roretzi*. In all pairwise combinations between 16 individuals (taken from a 10 m^2 plot), "contact reactions" were usually observed in allogeneic mixtures, but never in isogeneic ones. Although all individuals are produced sexually, not all individuals appear to be allotypically unique (at least to the extent that the "contact reaction" is sensitive to allotypic differences); in fact, 12 per cent (14/120) of unique allogeneic cultures proved compatible. Of equal importance is their observation that "contact reactions" are not predictable on the basis of transitivity. In several instances, one of two compatible individuals would be compatible with a third individual, whereas the other would not. As with *Botryllus*, not all allotypic differences are recognized as nonself. Subsequent, and more extensive, studies confirm these results (Fuke and Nakamura, 1985).

The only additional study of fusion/nonfusion frequencies in ascidians focuses on the social ascidian *Perophora sagamiensis* in which 4 of 16 stolon contacts produced fusions (Koyama and Watanabe, 1982). Although this is a rather high rate of compatibility, the sample size is so small and the propensity for asexual fragmentation is so high, that no sure assessment of levels of variability can be made.

Bryzoans

There are only two studies that address the existence of allorecognition specificity in bryozoans. In one study on *Thalamoporella californica*, Chaney (1983) showed that sibling colonies were generally fusible, whereas nonsiblings were not. Humphries (1979) provided similar, although less extensive, data for *Parasmittina nitida*.

Allotypic Frequencies and Spatial Distributions

A growing number of studies employ grafting, behavioral, and genetic data to quantify the dispersion and frequency of allotypes in natural populations. These data are necessary to examine assumptions about breeding structure and the relative importance of sexual versus asexual reproduction, as well as to test predictions of allotypic frequencies and spatial distributions. For instance, deviations from predicted equilibrium allotypic frequencies may be due to deviations from panmixia and have little to do with selection acting directly on allorecognition loci. Similarly, an excess of one allotypic class may be the result of selection favoring that allotype in a particular environment, or the result of a high frequency of clonal fragmentation of that allotype. The dispersion and frequency of allotypes also play important roles in determining the likelihood of contacts between compatible and incompatible individuals. In turn, the frequencies and consequences of fusion and behavioral cooperation versus rejection and aggression help to circumscribe the intensity of selection acting on allorecognition loci.

The dispersion of allotypes has been examined on a variety of spatial scales. Most studies comparing compatibility frequencies of individuals from sites separated by distances on the order of kilometers show low levels of compatibility (Jokiel, Hildemann, and Bigger, 1982; Curtis, Kerr, and Knowlton, 1982; Sebens, 1982; Fujii, 1987). In a highly fissile species of the gorgonian *Plexaura*, however, Lasker and Coffroth (1985) noted that individuals separated by about 1 km of uncolonizable habitat were often compatible. This result can be explained by low allotypic diversity or by the transport of clonal fragments between geographically isolated habitats (Lasker and Coffroth, 1985).

On finer spatial scales, a variety of evidence indicates that similar (or identical) allotypes are spatially associated. For example, Curtis, Kerr, and Knowlton (1982) found that the probability of graft acceptance in the sponge *Ectyoplasia ferox* decreased with increasing distance between grafts. Of eight attempted grafts between individuals initially separated by more than 100 m, none were compatible. Fusion frequencies increased with decreasing distance in such a way that individuals separated geographically by more than 2 m and less than 100 m fused in 6 of 17 cases. Fusion frequencies approached 50 per cent (47/96) in "allografts" between individuals taken within a 2 m^2 patch.

In a detailed study of allotypic dispersion in the sponges *Verongia longissima* and *Iotrochota*

birotulata, Neigel and Avise (1983a) observed patterns of allotypic association like those reported by Curtis, Kerr, and Knowlton (1982). Of 68 grafts between *V. longissima* separated by greater than 10 m, none were compatible. Of 105 grafts between *I. birotulata* individuals separated by greater than 2.7 m, none were compatible. A comparable analysis of two other sponges, *Aplysina fistularis* and *A. cauliformis*, gave no fusions in either 30 *A. fistularis* grafts separated by greater than 2.1 m or 12 *A. cauliformis* grafts separated by greater than 13 m (Neigel and Schmahl, 1984). At distances less than 2.1 m and 13 m respectively, they found numerous fusions, but assumed that these compatible grafts were between clonal fragments.

Neigel and Avise (1983b) used similar assays to document the dispersion of allotypes in a population of the extensively fragmenting coral *Acropora cervicornis*. They found once again that the probability of graft fusion decreased with increasing distance between grafted individuals. On a still finer spatial scale, however, patterns of allotypic association varied between two widely separated study sites. At one site near Discovery Bay in Jamaica, nearest neighboring colonies were unlikely to be the same allotype, whereas at another site near Tague Bay in St. Croix, neighboring colonies were frequently of the same allotype. These differences in neighborhood structure could reflect (1) the effects of different hydrodynamic regimes which strongly or weakly mix genets as they fragment, (2) site-specific historical differences in clonal diversity, and (3) population-specific differences in rates of asexual versus sexual recruitment (Neigel and Avise, 1983b).

Other studies indicate that dispersion of allotypes can deviate substantially from aggregations of similar allotypes. Wulff's (1986) thorough analysis of the spatial distribution of allotypes in three species of tropical sponge, *Iotrochota birotulata*, *Haliclona rubens*, and *Aplysina fulva*—one of which was studied by Neigel and Avise (1983b) at another location—provides little evidence that similar allotypes are spatially associated on any scale within a 200 m^2 study site. Similarly, behavioral and electrophoretic studies of the beadlet anemone *Actinia equina* (Quicke and Brace, 1983; Brace and Quicke, 1985, 1986a,b), and the aconti-ate anemone *Metridium senile* (Hoffmann, 1976), indicate that allotypes (or genotypes) are well mixed within local populations. The absence of any relationship between distance and the probability of graft compatibility (over a range of 1–12 m) in the coral *Montipora verrucosa* (Jokiel, Hildemann, and Bigger, 1983) also suggests that clonal structure can be weak in some populations.

The discrepancy between the findings of Neigel and Avise (1983b) and Wulff (1986) on population structure in *Iotrochota birotulata*, like the site-specific differences in clonal diversity found by Neigel and Avise (1983b), implies that even intraspecific patterns of allotypic dispersion and diversity can vary geographically. The importance of geographic variation is underscored by variation in clonal structure within and among populations of the anemone *Actinia tenebrosa*: clonal aggregation usually occurs on spatial scales consistent with those expected from limited dispersal of clonal propagules ($\cong$ 5–20 cm) and intergenotypic aggression ($<$ 2 cm) (Ayre, 1983). The magnitude of the aggregation, however, varies from site to site (Ayre, 1983, 1984a,b), and putative clonemates may be separated by up to 30 m of shoreline (Black and Johnson, 1979; Ayre, 1983). Taken together, these results indicate that environmental heterogeneity (e.g., distribution of favorable habitats, contour, flow regime) and historical differences in clonal diversity can strongly influence levels and patterns of allotypic variation quite independently of the selective and nonselective processes outlined previously.

Few studies comprehensively examine clonal diversity and allotypic frequencies over relatively large areas. Wulff's (1986) previously cited study of tropical sponges is the most complete. In a 10 m $\times$ 20 m quadrat, allotypic diversity was quite high in all species (*Iotrochota birotulata*—24 compatibility groups; *Haliclona rubens*—12 groups; *Aplysina fulva*—13 groups). Allotypic frequencies were strongly skewed, with one allotype (or a few) numerically dominating the populations of all three species.

INTERPRETATION OF ALLORECOGNITION ASSAYS: THE INFERENCE OF LEVELS AND SPATIAL PATTERNS OF ALLOTYPIC VARIATION

The need to infer levels and patterns of variation from allorecognition assays poses numer-

ous empirical ambiguities. In this section, I first identify some of these problems; I then evaluate the approaches usually used to infer the genetics of allorecognition and levels of allotypic polymorphism. Finally, I present a general interpretation of the formal genetics of allorecognition, and based on this interpretation, analyse levels and patterns of allotypic variation.

Empirical Problems

There are four classes of empirical problem that potentially confound the genetic interpretation of allorecognition assays. The first class of problem arises from the uncertainty of knowing a priori whether a graft is an allo- or isograft. For aclonal organisms, grafts between physiologically discrete individuals must be allografts. If allograft fusions are rare among members of panmictic populations, levels of allelic variation for allotypic determinants may be high, or many loci may control allorecognition (Curtis, Kerr, and Knowlton, 1982). If allograft fusions are relatively common among aclonal organisms, then there are at least three potential explanations: (1) many individuals share allotypes because there is little allelic variation, (2) the allorecognition system may not distinguish among all allelic variants, or (3) allotypic differences may be recognized, but incompatibility reactions are not initiated.

The interpretation of grafting and behavioral data is still more complex in clonal organisms because a given genotype may be represented in a population by a number of physiologically discrete individuals. As with aclonal organisms, high rates of allograft acceptance may result from either low levels of variation for allorecognition determinants, or imperfect sensitivity of recognition (or effector) mechanisms to allotypic differences. In addition, allografts between ramets in clonal organisms may not be allografts at all, but rather autografts between asexually produced ramets of the same genet. High rates of graft acceptance may therefore reflect high frequencies of asexual versus sexual proliferation, or local retention of asexual propagules (Hughes and Jackson, 1982, 1985; Neigel and Avise, 1983a,b).

The relative frequency of asexual multiplication versus sexual reproduction in clonal organisms has four important consequences for the study of variation at allorecognition loci. First, this frequency can determine how many fusions are due to interactions between genuinely allogeneic individuals, and how many fusions result from autografts between physiologically discrete, but isogeneic, individuals. Second, if some genotypes in a population are represented by more clonal replicates than other genotypes, estimates of allotypic frequencies may reflect the propensity of particular genotypes to proliferate asexually, rather than the direct actions of evolutionary processes on allorecognition loci. Third, this ratio influences patterns of allotypic variation, for high rates of sexual reproduction will rearrange allotypes through syngamy and recombination. Fourth, the relative importance of sexual versus asexual recruitment into a population can dramatically affect equilibrium levels of allotypic variation (Neigel and Avise, 1983b)

The second class of problem is a matter of sampling scale, and how the dispersal distances of sexual and asexual propagules determine the appropriate scale over which histocompatibility assays should be performed. The spatial distribution of allotypes—which will depend upon adult movement, dispersal of sexual and asexual propagules and gametes, habitat suitability, and historical events—is a critical datum for testing the predictions of the population genetic models. For example, the spatial arrangement of allotypes will control the likelihood of interactions between compatible and incompatible individuals (Stoddart, 1988). Scale effects can also profoundly affect the reckoning of variation in a population. If dispersal of sexual and asexual propagules is extensive, then comparatively small areas will carry a fairly complete sample of the allotypic diversity in a population. In contrast, a spatially limited study may underestimate the range of variation within a population if dispersal of asexual or sexual propagules is restricted.

Differences in mobility among different types of dispersing propagules [e.g., asexually versus sexually produced—see Jackson (1985, 1986)], coupled with differences in intensity of asexual multiplication versus sexual reproduction, will further affect dispersion of allotypes (e.g., Lasker and Coffroth, 1985; Wulff, 1986). Consider the following example: Jokiel, Hildemann, and Bigger (1982) found no

fusions in grafts between sponges taken from isolated patch reefs. They inferred that there was a high degree of allotypic variation. Given their demonstration that within-reef grafts rarely fused, their conclusion appears to be sound. Without such small-scale grafting data, however, the absence of fusion in grafts between individuals from different reefs could be explained by a single fixed allelic difference, provided there is little gene flow between the reefs. In general, so little is known about the dispersal distances of asexual and sexual propagules, and the relative frequency of asexual versus sexual propagation, that it remains difficult to estimate levels of gene flow between spatially discrete populations.

Third, there is some evidence that the capacity for allorecognition changes ontogenetically, and that compatibility relationships may be temporally unstable. Hidaka (1985) found no compatible combinations of adults in the coral *Pocillopora damicornis*; however, juveniles from incompatible adults fused to form chimeras. Several other recent studies on colonial ascidians and cnidarians raise three flags that serve as warnings for cautious interpretation of grafting studies. Sabbadin (1982) observed that repeated grafts between genetically compatible colonies of the ascidian *Botryllus schlosseri* usually fuse, but occasionally display the classical signs of rejection [also see Lasker and Coffroth (1985) for a similar example in gorgonians]. Additionally, *Botryllus* allografts may initially fuse, but subsequently reject each other (Scofield et al., 1982; Rinkevich, pers. commun.). In contrast, initial allogeneic contacts between anemones usually lead to aggression (i.e., incompatibility); however, in *Metridium senile* (Purcell and Kitting, 1982) and *Anthopleura elegantissima* (Sebens, 1984), aggressiveness declines after extended allogeneic contact. A few studies also indicate that in some fused allogeneic pairs, one colony appears to be resorbed by the other (L. W. Buss, A. Sabbadin, and V. L. Scofield, pers. commun.; Rinkevich and Weissman, 1987a, b). Whether the physical resorption of one fusion partner carries with it the disappearance of its genotype remains to be seen; however, it is a distinct possibility that chimeras are genetically unstable.

Finally, the existence of intraspecific geographic and temporal variation in levels of allotypic diversity potentially undermines the reliability of generalizations based on single samples in space and time. Sebens (1982) calculated that clonal diversity in the anemone *Anthopleura elegantissima* ranged from 1 to 25 clones per 100 m^2 at different sites along the coast of Washington. In the colonial ascidian, *Botrylloides violaceous*, Mukai and Watanabe (1975b) found that 16 per cent (31/190) of the pairings were fusible when all pairings from two nearby sites were pooled; however, within site 1 there was a fusion frequency of 8.9 per cent, whereas site 2 had a significantly higher fusion frequency of 28.9 per cent (test for equality of two percentages; $p < 0.02$). Curiously, between-site grafts fused more often than within site 1 grafts, although the difference is not statistically significant ($p > 0.2$). This study, along with data on a closely related ascidian, *Botryllus primigenus* (Mukai and Watanabe, 1975a), also indicates that there can be significant variation in compatibility frequencies between years at a given site.

Theoretical Problems

The major theoretical difficulty with using histocompatibility assays to estimate levels of variation is well illustrated by several simple models from Curtis, Kerr, and Knowlton (1982). Consider a panmictic population at linkage equilibrium, with all alleles at any given allorecognition locus equally frequent. Further assume that there is no asexual proliferation (i.e., each individual is a separate diploid product of sexual reproduction). In such a population, three parameters will control the probability of compatibility between two randomly chosen individuals: (1) the number of independent loci segregating for the allorecognition trait, (2) the number of alleles segregating at each locus, and (3) the genetic rules governing compatibility (i.e., complete matching of allotypic determinants, or some form of partial matching). If complete genetic matching is required for compatibility, then according to Curtis, Kerr and Knowlton (1982) the probability that two individuals will be compatible is predicted by:

$$[(2n - 1)/n^3]^L, \qquad (10)$$

where n is the number of alleles per locus, and L is the number of independent loci. If only one allele per independent locus need be

shared for compatibility, then the probability of compatibility increases to:

$$[(4n^2 - 6n + 3)/n^3]^L. \quad (11)$$

Fig. 4 shows these probabilities for one-locus models of partial and complete genetic matching, and indicates two difficulties of using histocompatibility tests to assess levels of variation at allorecognition loci. First, compatibility frequencies can only weakly discriminate between the complete and partial genetic matching hypotheses unless far more assays are performed than there are individuals in the population. This problem is especially onerous if multiple loci control histocompatiblity. Second, compatibility assays alone do not allow estimates of allelic variation. For example, low fusion frequencies in panmictic populations may reflect high levels of polymorphism at one or a few loci. Alternatively, low levels of polymorphism at many unlinked loci, or restrictive rules of genetic matching, could produce results similar to those predicted by single-locus models.

Although the model of Curtis, Kerr, and Knowlton (1982) is based on some potentially unrealistic assumptions, the general result is robust: the genetics of allorecognition and levels of variation cannot be directly inferred from compatibility frequencies alone. Furthermore, if both asexual propagation and sexual reproduction occur in a population, it is potentially circular to assume that fusible individuals are clonemates, then use histocompatibility assays to estimate the relative frequencies of asexual versus sexual recruitment into the population. Without independent characterization of genetic identity, it is virtually impossible to ascertain what fraction of compatible pairings is due to isogeneic grafts between clonal fragments, and what proportion of compatible matches is due to allogeneic grafts among siblings, half-siblings, or more distantly related individuals.

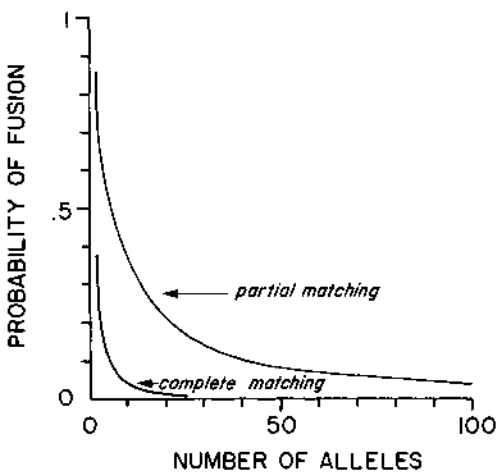

FIG. 4. THE PROBABILITY OF INTERGENOTYPE FUSION VERSUS THE NUMBER OF ALLORECOGNITION ALLELES IN A POPULATION
The details of the model are given by expressions (10) and (11). The curves depict predictions for single-locus allorecognition systems with partial and complete genetic matching.

Rules of Allotypic Matching

As the previous section demonstrated, knowledge of the genetical rules of allotypic matching is critical to estimating levels of allotypic variation based on allorecognition assays. For example, if it is assumed that a single locus controls allorecognition and only one allele need be shared for compatibility, an observed compatibility frequency of 4 per cent would require approximately 100 equally frequent alleles. In contrast, if full matching were required, somewhere between 6 and 7 alleles would give a comparable frequency of compatibility.

In the absence of formal genetic analysis, two approaches have been used to answer the question, Do the results of allorecognition assays accurately reflect allotypic diversity within populations? One approach, advocated by Hildemann and his colleagues (e.g., Hildemann, Johnston, and Jokiel, 1979; Jokiel, Hildemann, and Bigger, 1982), holds that groups of mutually compatible individuals each represent a clone, or a single allotype. As a corollary, high levels of compatibility are taken to represent a high rate of asexual propagation, and not low levels of polymorphism. I, and others (e.g., Jokiel, Hildemann, and Bigger, 1982), term this view, following Medawar (1981), the "uniqueness of the individual" concept. If this concept is accepted, then the number of mutually compatible groups of individuals in a population reflects variation at allorecognition loci. Nonetheless, the concept does not state how the variation is partitioned: there may be many alleles at one locus (or a

few loci) or few alleles at many independent loci. Rather, the concept deals with operational expectations and merely provides a reflection of the overall complexity of the genetics of allorecognition.

The alternative to the "uniqueness of the individual" concept holds that at least some allogeneic individuals are compatible. Thus, compatible individuals are not necessarily clonemates. This view does not specify whether allogeneic individuals are compatible because they share all allotypic determinants (as some fraction of siblings would), or if only partial matching of allodeterminants is required for compatibility. If only partial matching is required for compatibility, then allorecognition assays would underestimate levels of genetic variation, because some individuals would be compatible without sharing all allodeterminants.

The "uniqueness of the individual" concept is based on two implicit assumptions: (1) compatible individuals must share all determinants at allorecognition loci; and (2) levels of variation, whether the variation is partitioned within or among loci, must be sufficiently high that only clonemates will share all allotypic determinants (i.e., will have complete genetic matching). The strength of the "uniqueness of the individual" concept and its corollary, the complete genetic matching hypothesis, rests on three lines of evidence. First, low rates of compatibility, particularly between spatially separated individuals, imply that levels of variation for allodeterminants are high. The validity of this inference, however, depends on the spatial scale of dispersal of asexual and sexual propagules and the area from which grafts were taken. For example, in one study of the coral *Montipora*, individuals from three geographically separate populations were grafted and fusions were exceptionally rare (Hildemann, Jokiel, Bigger, and Johnston, 1980). If full matching were required for fusion, a single allelic difference at a compatibility locus would be sufficient to prevent self-recognition and fusion. Therefore, a three-allele polymorphism, with each source population being fixed for a different allele, could produce the same paucity of fusions.

Second, the probability of compatibility between individuals often attenuates with increasing distance between assayed individuals (e.g., Jokiel, Hildemann, and Bigger, 1982; Neigel and Avise 1983a,b; Neigel and Schmahl, 1984). This line of evidence supposes that dispersal of asexual propagules is spatially restricted, and either that (1) individuals arising from sexual propagules are incompatible or (2) sexual propagules are spread so widely that they rarely come into contact. Thus, compatibility among proximate individuals presumably reflects isogeneic grafts between clonal fragments. If it were true that sexual propagules consistently dispersed farther than asexual propagules, then the pattern of attenuating fusion frequencies with distance would be consistent with the "uniqueness of the individual" concept. Jackson (1985, 1986) and Grosberg (1987), however, have recently reviewed evidence suggesting that the sexually produced larvae of many clonal benthic invertebrates often disperse within a few meters of their birthplace. Compatibility between proximate individuals may therefore reflect either compatibility between clonemates, or between sexually produced sibling larvae which share some, or all, of their allotypic determinants.

The third line of support for the "uniqueness of the individual" concept is based on the observation of complete transitivity of compatibility relationships among trios of apparently allogeneic sponges (Kaye and Ortiz, 1981; Neigel and Avise, 1983a,b, 1985; Neigel and Schmahl, 1984; Wulff, 1986). I have discussed in an earlier section (p. 389) some of the problems with using transitivity tests to infer the genetics of allorecognition. In any case, observations of compatibility between putatively allogeneic individuals appear to refute the generality of the "uniqueness of the individual" concept. In two controversial studies, Burton (1949) and Borojevic (1967) suggested that sexually produced conspecific sponge larvae may sometimes fuse. Other studies on freshwater sponges indicate that fusion of conspecific juveniles can be common (Ankel and Eigenbrodt, 1950; Rasmont, 1970).

Not all sponge biologists agree that postlarval fusion involves allogeneic individuals. Johnston and Hildemann (1982) disputed Borojevic's claim, in particular, and other reports of allogeneic fusions, as well, partly because of their belief that sexually produced individuals ought to be sufficiently distinct at allorecognition loci that they would not be

fusible. By this reasoning, the post-larval fusions discussed above may have involved asexually produced, hence isogeneic, larvae. In sponges, there is little evidence that asexually produced propagules resemble sexually produced larvae (Bergquist, 1978). Several recent genetic studies, however, indicate that apomictic parthenogenesis occurs in some anthozoan cnidarians (e.g., Black and Johnson, 1979; Orr, Thorpe, and Carter, 1982; Stoddart, 1983). In this light, observations of fusion between siblings such as those on the coral *Pocillopora* (Stephenson, 1931) and the athecate hydroid *Hydractinia echinata* (Teissier, 1929) must be interpreted cautiously. Nonetheless, apomictic parthenogenesis cannot readily explain observations of frequent fusion among sibships of larvae from incompatible adult strains of the coral *Pocillopora damicornis* (Hidaka, 1985) or the occurrence of fusion masses composed of larvae from sites separated by tens of kilometers in the demosponge *Ophlitaspongia seriata* (Fry, 1971).

Where independent means have been used to assess the identities of genotypes, the results are often at odds with the "uniqueness of the individual" concept and the requirement of full genetic matching (e.g., Ayre, 1982; Heyward and Stoddart, 1985; Neigel and Avise, 1985; Resing and Ayre, 1985; Willis and Ayre, 1985; but see Hunter, 1985). Numerous breeding studies also indicate that allogeneic individuals may be compatible (e.g., Hauenschild, 1954, 1956; Oka and Watanabe, 1960; Sabbadin, 1962; Van de Vyver, 1970; Van de Vyver and Willenz, 1975; Ayre, 1982; Sabbadin, 1982; Neigel and Avise, 1985; Heyward and Stoddart, 1985, and references therein). Moreover, even if complete genetic matching were required for compatibility, some fraction of close relatives ought to share a full complement of allodeterminants (depending, of course, on the number of segregating loci).

The Number of Loci Controlling Allorecognition

A high degree of allotypic specificity could be conferred by a continuum of underlying genetic variation ranging from one locus with many alleles to many independent loci with just a few alleles (Curtis, Kerr, and Knowlton, 1982). The fact that close relatives are often compatible provides some insight into where allotypic variation lies along the continuum from a single locus to multiple loci. First, assume that two individuals mate, each heterozygous for different allorecognition alleles. These parents would therefore be incompatible. Further assume that complete genetic matching is required for individuals to be compatible. For each independently segregating locus that controls allorecognition, the probability that two full-sibs will share a maternal allele is 0.5, and the probability that they will share a paternal alleles is likewise 0.5. Therefore, the probability that they will share both alleles is 0.5×0.5 or 0.25 per locus. For a one-locus system, 25 per cent of full-sibs should be compatible. If only one allele need be shared for compatibility (i.e., partial matching), then for a one-locus system, 75 per cent of full-sib pairings would be compatible. As the number of independent loci controlling allorecognition increases, the likelihood of allelic matching at all loci will decrease exponentially according to the number of loci. For example, if the probability of two full-sibs sharing two alleles at a locus is 0.25, then the probability of their sharing both alleles at five loci is 0.25^5 or 10^{-3}. If only one allele need be shared, then the probability increases to 3.13×10^{-2}. This example, in combination with the few formal genetical data available showing that progeny usually fuse with their parents and siblings, implies that the loci controlling allorecognition are unlikely to be numerous and spread across many chromosomes.

Levels of Allotypic Variation in Natural Populations

The results of allorecognition assays on a wide variety of taxa generally indicate that compatibility is rare, particularly between individuals separated by more than a few meters. As the considerations in the previous subsections demonstrate, the amount of allotypic variation required to produce such low levels of compatibility depends strongly upon assumptions about the number of loci controlling allorecognition, and the genetic rules of matching.

Given the single-locus, partial matching *Botryllus* genetic model, and assuming that (1) all alleles are equally frequent, (2) rates of asexual proliferation can be estimated in order to correct for fusions between syngeneic clonal fragments, and (3) the population is panmic-

tic, it should be possible to estimate the number of alleles in a population at Hardy-Weinberg equilibrium by solving expression (11) using the results of grafting assays. The first assumption is troublesome and difficult to examine empirically. My own data (Grosberg, 1982, 1987) suggest that *Botryllus schlosseri* colonies in Woods Hole rarely propagate asexually; whether this is a general attribute of *Botryllus* biology remains to be seen. The third assumption is clearly violated in Woods Hole populations of *Botryllus schlosseri*: most larvae settle within a few meters of their birthplace (Grosberg and Quinn, 1986; Grosberg, 1987). This certainly leads to spatial association of relatives, and consequently higher probabilities of fusion between proximate individuals than would be expected if dispersal were extensive. Nevertheless, if samples are taken over an extensive area, the bias introduced by limited sibling dispersal may be somewhat circumvented.

If one assumes an average fusion frequency of about 5 per cent in natural populations of *Botryllus*, solution of the partial matching expression (11) for one locus suggests that there are at least 80 alleles. The number of unique genotypes at a locus is defined by expression (7); using $n = 80$ gives 3240 unique histocompatibility genotypes and an expected fusion frequency of 0.03 per cent with complete matching.

In the absence of formal genetic data from other taxa, it is difficult to encapsulate the remaining data on compatibility frequencies. However, several generalizations hazily emerge. Perhaps the most important is that invertebrate allorecognition mechanisms appear to be remarkably efficient at discriminating between self and nonself genotypes. Even in taxa and populations where allorecognition does not perfectly distinguish among genotypes, recognition "errors" are likely to be confined to encounters involving closely related kin.

With the exception of botryllid ascidians, it remains impossible to specify exactly how many loci are involved in allorecognition, hence to infer precisely levels of allelic variation. Nonetheless, the data analysed in the previous section suggest that most allotypic variation resides at relatively few loci. On the one hand, this is a heartening observation, for it suggests that understanding the formal genetics of allorecognition may not be an insurmountable task once technical problems are stripped away. On the other hand, this tentative conclusion, in combination with the usual pattern of highly precise allorecognition, poses the difficulty of how allelic variation is maintained.

Spatial Distribution and Frequency of Allotypes

The data generally suggest that allotypic diversity is usually quite high within a limited geographical area (a population?), and that similar or identical allotypes are often spatially associated. There is comparatively little information about the relative frequencies of allotypes in populations, but the studies of Sebens (1982) on anemones and Wulff (1986) on sponges suggest that one allotype, or a few, can dominate samples.

To distinguish among theoretical predictions, it is imperative that the causes of spatial association of similar allotypes and unequal allotypic frequencies be understood. Spatially varying selection in which specific genets are favored in particular patches could underlie association of similar allotypes (e.g., Ayre, 1985; Brace and Quicke, 1986b). The same pattern of aggregation, however, could be the result of limited dispersal of clonal fragments or of sibling sexual propagules from a source genet (e.g., Grosberg and Quinn, 1986). Even if it could be shown that spatial association of similar allotypes was the result of spatial variation in selection, such a pattern could result from selection acting on components of the genotype other than the allotype.

Similar alternatives exist for explaining the observation that allotypic frequencies are often unequal: selection could be favoring particular genets or allotypes; however, genet-specific variation in rates of asexual proliferation (e.g., Sebens, 1982; Stoddart, 1984; Brace and Quicke, 1986a,b; Wulff, 1986) or levels of aggression (Francis, 1973a,b; Purcell, 1977; Ottaway, 1978; Ayre, 1983, 1987) could also produce unequal genet, hence allotypic, frequencies.

The determination of whether spatial and frequency distributions of allotypes are patterned in a specific way will be quite sensitive to the spatial extent of a sample. For example, on sampling scales that just encompass the area of one clone, or a few, similar allotypes

will appear to be highly aggregated, and the sample will be dominated by a small number of allotypes. Over larger spatial scales, spatial patterns may appear to be more random, and relative allotypic frequencies may equalize. In evolutionary terms, the critical spatial scale would minimally encompass the interbreeding population.

THE EMPIRICAL DATA AND THE THEORETICAL ISSUES

Without precise estimates of effective population size, mutation rates, migration rates, number of alleles, and allelic frequencies, it is impossible to test rigorously any of the five genetic theories outlined earlier. In addition, a variety of factors other than selection, drift, and mutation are known to influence allotypic dispersion and diversity. These factors include the structural and hydrodynamic attributes of the environment (e.g., Wulff, 1986), the frequency of physical and biological disturbance (e.g., Neigel and Avise, 1983b), the relative contributions of sexual and asexual recruitment to population growth (Neigel and Avise, 1983b), and the allotypic diversity carried by the individual (or individuals) that founded a population (Parker, 1979; Neigel and Avise, 1983b). The intrusion of these variables may also prevent a population from reaching evolutionary equilibrium (Neigel and Avise, 1983b).

The power of the neutral allele theory to account for allotypic polymorphism depends explicitly on effective population sizes and mutation rates. Such data are not available for any of the taxa discussed in this review. If allorecognition is controlled by partial matching at a single locus (e.g., *Botryllus*), then to account for levels of graft acceptance between 2 per cent and 5 per cent one is faced with explaining at a minimum the maintenance of between 80 and 110 alleles at equilibrium. [If the alleles were not equally frequent, then more alleles would be needed to explain the observed graft acceptance rates (Wright, 1939).] Unless one is willing to invoke either extraordinarily large population sizes or mutation rates, neutral allele models seem incapable of maintaining such high levels of variation. If, however, full genetic matching were a requisite for compatibility, or more loci were involved in allorecognition, the number of alleles necessary to maintain a given level of specificity drops considerably. This seems to revive hopes for the neutral models; however, the theoretical problem is more complex than it appears, for one needs to explain why alleles do not drift to fixation in small populations such as those studied by Wulff (1986).

The power of verbal arguments invoking heterozygous advantage is severely limited by mathematical considerations (Lewontin, Ginzburg, and Tuljapurkar, 1978). Only in very specific (and improbable) genetic circumstances could heterozygote advantage maintain more than a few alleles at a locus. That such genetic circumstances should be found in a diverse array of phylogenetically and demographically distinct taxa seems so improbable that single-locus models of heterosis should probably be eliminated from consideration. Even if allotypic variation were distributed more evenly among loci, heterosis remains a problematical explanation because I have assumed that the loci regulate a similar function, namely allorecognition. Thus, although the loci are not mechanically linked on chromosomes, they are functionally linked by epistasis. I am unaware of multiple-locus models of overdominance that incorporate epistatic interactions, but it seems likely that if epistasis occurs, heterosis will not be a strong candidate for the maintenance of multiple-locus polymorphism.

The model capable of maintaining the most variation with the fewest assumptions involves some form of frequency-dependent selection. Even in relatively small populations, and assuming conventional mutation rates, high levels of allelic variation can be maintained. For example, assuming that there are 30 alleles in a population, solving equation (4) shows that a new variant will carry a 10 per cent selective advantage over alleles already extant.

If frequency-dependent selection operates in a manner consistent with the gametophytic incompatibility systems of angiosperms, then at equilibrium, all compatibility alleles should be equally common. For natural populations of clonal benthic invertebrates, there are no conclusive data favoring or opposing this prediction. For allorecognition loci in mammalian populations, most evidence suggests that allelic frequencies are far from equal both within and among populations (Bodmer and Bodmer, 1978; Wakeland and Nadeau, 1980; Reimann and Miller, 1983). Some have interpreted these discrepancies between observed and expected

frequencies as favoring either nonselection models (e.g., Reimann and Miller, 1983), or selection models in which specific alleles are favored in particular environments or populations (Bodmer and Bodmer, 1978; Klein, 1979, 1982; Wakeland and Nadeau, 1980).

For low vagility organisms, there is little doubt that the environment is heterogeneous in terms of selection, perhaps even on very fine spatial scales. Given, however, that allelic diversity depends on the number and frequency of selectively different patches, if the organisms themselves were the major source of selection on allorecognition loci, then spatially variable and frequency-dependent models could be considered as two sides of the same coin. For example, individual fitness in several grasses is known to be inversely dependent on kinship of neighbors (reviewed in Bell, 1985). Similarly, the strength of selection favoring the evolution of pathogen resistance will be the result of a balance between the fitness differences of resistant versus nonresistant forms and the intensity of selection (Levin, 1986). In turn, this intensity will depend on the spatial distribution of the selection agent (i.e., can susceptible individuals leave an infected patch?) and the frequency of patches that contain the pathogen. The overall frequency of resistant versus nonresistant varieties will therefore depend upon (1) the fitness costs of being infected versus being resistant, (2) the frequency of patches with the pathogen, (3) the frequency of pathogens within the patches, and (4) the transmissibility of the pathogen.

A frequency-dependent model, perhaps in combination with heterosis, would be the most powerful approach to analysing the evolution of pathogen resistance under these circumstances, particularly if the spatial distribution and frequency of specific pathogens changes from host generation to host generation (Gillespie, 1975; Seger and Hamilton, 1988). Similarly, if intraspecific interactions between allotypes determine the fitnesses of different allotypes, and if the spatial distribution of allotypes changes from generation to generation, then although selection undoubtedly varies spatially within generations, the distribution of "patches" varies across generations. Thus, frequency-dependent models would be the most appropriate analytical approach.

It is premature to accept or discard any of these hypotheses. In the case of frequency-dependent selection, it is only with systems in which alleles have no function other than to control compatibility that equal-frequency equilibrium conditions will occur. Whether populations are in genetic equilibrium with respect to (1) breeding structure, (2) natural selection, (3) mutation, and (4) drift is an open question on all counts. Indeed, it would be surprising to find that the genetic architecture of any natural population conformed exactly to theoretical predictions. Moreover, the assumption that the phenotypic effects of allorecognition loci are limited to allorecognition specificity is probably a great oversimplification.

MECHANISMS MAINTAINING ALLOTYPIC VARIATION

Any useful theoretical explanation for the maintenance of allotypic polymorphism must include a plausible mechanism. Measurements of the genetic attributes of a population can only be consistent or inconsistent with a theoretical prediction; they do not provide a mechanism by which the theory operates. Neutralist models assume that allotypic variation has no functional significance, and the burden is to show that this is so. Selectionist models bear the opposite burden. Thus, advocates of both selectionist and neutral theories would likely agree with the statement, "A consequence of allotypic variation is to confer specific identities on genotypes within populations." Neutralist models would assume, however, that individuality is of no selective importance. In contrast, selectionist models, particularly those invoking frequency-dependent selection, would assume that allotypic variation is the result of natural selection favoring individuality.

Two conditions must be met for natural selection to maintain allotypic variation by restricting fusion or aggressive behavior. First, fusion/nonfusion or agonistic behavior that is conditioned on allotype must be selectively advantageous. Second, novel allotypic variants must be favored and maintained. In this section, I explore some of the phenotypic consequences of allorecognition, emphasizing how selection may act on allorecognition loci.

The Control of Fusion

In groups such as hydroids, sponges, and colonial ascidians where contacts between compatible individuals lead to clonal fusion

and chimera formation, the absence of allotypic variation would lead to genotypically unrestricted fusion. In this section, I examine some of the potential benefits and costs of fusion between genotypes.

There are several ecological and demographic circumstances in which fusion between clones may provide substantial fitness benefits (reviewed in Buss, 1980; Jackson, 1985; Grosberg and Quinn, 1986). First, because members of small size classes are particularly susceptible to mortality (e.g., Wulff, 1986), fusion between small individuals immediately increases the total size of the chimera, and perhaps survivorship, without the delay required for growth (Buss, 1982; Jackson, 1985; Hughes and Jackson, 1985). Where mortality is size-dependent, one fusion partner or both of them (depending upon their relative sizes) may gain survival benefits. Hidaka (1985) even showed that juvenile corals fused more readily than conspecific adults. Second, the initiation of sexual reproduction often depends upon an individual reaching a minimum critical size (van Duyl, Bak, and Sybesma, 1981; Buss, 1982; Wahle, 1983; Winston and Jackson, 1984; Jackson, 1985; Kojis and Quinn, 1985; Szmant-Froelich, 1985; reviewed in Harvell and Grosberg, in press). Colony fusion permits more rapid attainment of that size, and consequently may lower age at first reproduction. In a growing population, or where post-juvenile mortality is high, such a decrease in age at first reproduction may yield a substantial gain in fitness (Lewontin, 1965; Gadgil and Bossert, 1970; Schaffer and Gadgil, 1975; Charlesworth, 1980; Buss, 1982). Third, fusion potentially leads to the mixing of cells from two genotypes. Just as asexual fragmentation can decrease the risk of genotype extinction by spreading clonal replicas through space, colony fusion could decrease the risk of genotype extinction by spreading a genotype through a larger soma. This may be an especially important benefit in clonal organisms that can suffer partial mortality.

There are, however, potential costs to fusion, and the resulting mixing of genetically distinct cell lines. In most clonal taxa, a line of stem cells retains the competency to differentiate into either gametes or somatic tissue throughout the life cycle (Berrill and Liu, 1948; Nieuwkoop and Sutasurya, 1981; Whitham and Slobodchikoff, 1981; Buss, 1982). Sabbadin and Zaniolo (1979) showed that vascular fusion between allogeneic colonies of *Botryllus schlosseri* led to the free exchange of germ cells across the former boundary between the two colonies. Under these conditions, one member of a chimera could parasitize the other (Buss, 1982, 1983). For instance, one individual composing a chimera would gain a disproportionate share of gametic output by restricting the differentiation of its stem cells to gametic pathways while using the somatic tissues of the other member of the chimera for maintenance as well as for provisioning ova and for brooding embryos (Buss, 1982, 1983). Displacement of one cell lineage at the expense of another is known in chimeras of fungi (Davis, 1959; Pittenger and Brawner, 1961); myxomycetes (Clark and Collins, 1973); cellular slime molds (Buss, 1982); sponges (Van de Vyver, 1988); and colonial ascidians (Sabbadin and Zaniolo, 1979).

The simple physiological union of two genotypes may also pose substantial fitness costs to one or both members of a chimera. Rinkevich and Loya (1983) showed that there is oriented translocation of photosynthate in coral chimeras. In addition, fusion provides a direct path for the transmission of pathogens (Buss, 1982). Recently, Rinkevich and Weissman (1987a,b) demonstrated that resorption of the soma of one member of a fused pair can occur in *Botryllus schlosseri*.

It appears intuitively that individual selection acting to restrict allogeneic fusion could represent a general and potent selection force favoring the evolution of allorecognition and allotypic specificity (Burnet, 1971, 1973; Buss, 1982). In a theoretical analysis of this problem, Grosberg and Quinn (1988) defined the conditions necessary to favor rare allotypic variants in a single-locus, haploid model. Let c_f be the net per-capita fitness cost of fusion and b_f be the net fitness gain that is due to fusion. The expected fitness of an allorecognition allele, i, upon which fusion is conditioned, is then

$$W_i = 1 + P_i(b_f - c_f), \qquad (12)$$

where P_i is the frequency of allele i. Equation (12) shows that the fitness of an allorecognition allele depends upon both its frequency and the relative costs and benefits of fusion. If b_f

is greater than c_f, then W_i will increase as P_i increases, and the allele will become fixed in the population. However, if c_f is greater than b_f, then as P_i increases, W_i decreases. Consequently, rare alleles will be favored and allotypic variation will accumulate only when the costs of fusion exceed the benefits. This raises the question of why individuals should ever fuse (Grosberg and Quinn, 1988).

One of the important effects of high levels of allotypic variation is the restriction of fusion to closely related individuals. Although the costs and benefits of genotype fusion should be adjusted according to the relatedness of the fused colonies (Hamilton, 1964; Buss and Green, 1985; Grosberg and Quinn, 1986), the effects of kin selection have not yet been incorporated into mathematical models of the evolution of allotypic specificity.

The Control of Agonistic Behavior

Virtually all cnidarians possess nematocytes, highly specialized cells that contain extrudable, often toxic, organelles, the nematocysts (Mariscal, 1974). Nematocysts are used to capture prey and to deter predators (Mariscal, 1974; Buss, McFadden, and Keene, 1984). Nematocyst discharge may also be stimulated by allogeneic contacts; nonself tissues are often damaged, and competitively inferior individuals may suffer decreased growth or reproductive output, or be overgrown and killed (see references in Bigger, 1980; Ayre, 1983; Buss, McFadden, and Keene, 1984). For example, allogeneic contacts between adult colonies of the athecate hydroid *Hydractinia echinata* often lead to the production of swollen, nematocyst-laden, hyperplastic stolons (Schijfsma, 1939; Müller, 1964; Toth, 1967; Ivker, 1967, 1968, 1972). In most cases, one of the two interacting strains eventually overgrows and kills the other (Ivker, 1972; Buss, McFadden, and Keene, 1984). The outcomes of such agonistic intraspecific encounters are potentially of great ecological importance, for they can influence the ability of a clone to maintain, or expand, the space it occupies (Francis, 1973a,b; Sebens, 1982; Ayre, 1983; Buss, McFadden, and Keene, 1984).

In groups such as anemones and corals in which allorecognition systems control the initiation of aggressive or defensive behavior and the deployment of agonistic structures, the absence of allospecificity would lead to either no aggression or universal aggression. If universal aggression were the rule, then indiscriminate aggression against self would appear to be evolutionarily unstable (*sensu* Maynard Smith, 1982) in the face of a strategy that restricted aggression to unrelated individuals. Alternatively, if the absence of allotypic polymorphism led to no aggression, then there would be no costs associated with deploying and being damaged by aggressive or defensive structures. In this situation, individuals possessing rare alleles would face a disproportionately large fraction of aggressive interactions compared to individuals carrying common alleles, hence, the rare allorecognition alleles required to confer allotypic specificity would be selected against (Crozier, 1986).

In a model similar in form to their fusion analysis, Grosberg and Quinn (1988) demonstrated that the fitness of an allorecognition allele, i, that controls aggression, as a function of its frequency, P_i, is defined by

$$W_i = 1 - c_a + b_a/2 + c_a(P_i), \quad (13)$$

where W_i is the fitness of allele i, c_a is the per-capita cost of aggression, and b_a is the per-capita benefit of aggression. Equation (13) shows that W_i increases linearly with P_i as long as there is some positive cost to aggressive behavior. The cost/benefit differential has no effect on this relationship; thus, rare allorecognition alleles will be selectively excluded and allotypic polymorphism will not accumulate. Even if it were advantageous to direct aggression toward non-kin and away from kin, the polymorphism underlying allotypic specificity will not evolve.

In this simple model, aggressive behavior does not provide the selective impetus to maintain allotypic variation. Buss, McFadden, and Keene (1984) suggested that the fitness costs of fusion could maintain polymorphism in the context of aggressive behavior. Grosberg and Quinn (1988) have modelled this situation for organisms such as the hydroid *Hydractinia echinata* in which conspecific interactions lead either to fusion between compatible genotypes or aggression between incompatible genotypes. Their analysis indicates that a model combining fusion and aggression can maintain allotypic variation, provided the net benefit of fusion is less than that of aggression.

Pleiotropic Effects of Allorecognition Loci

There is growing evidence that allorecognition loci have phenotypic consequences that extend beyond the control of colony fusion and intergenotypic aggression (Blaustein, 1983; Jones and Partridge, 1983). Given such pleiotropic effects, allotypic variation could be maintained by frequency-dependent selection acting on traits other than individual specificity (Neigel, 1988). Two pleiotropic models for which there is some support include the control of gametic compatibility and the susceptibility to pathogenic infection.

The major line of support for gametic compatibility models comes from studies on the hermaphroditic colonial ascidian *Botryllus primigenus*. The ovum of *Botryllus* is surrounded by a diploid, maternally produced follicle. Oka (1970) reported that sperm sharing an allorecognition allele with the follicle are unable to fertilize the egg within. Scofield et al. (1982) provided similar evidence for an allorecognition-based block to fertilization in their studies of a Monterey Bay population of *B. schlosseri*. Such a fertilization block caused by the allorecognition mechanism would result in frequency-dependent selection acting in a manner analogous to angiosperm gametophytic incompatibility systems. Although such a pleiotropic effect is a plausible mechanism for maintaining allotypic polymorphism, data on other populations of *Botryllus*, as well as of the solitary ascidian *Halocynthia roretzi* (Fuke, 1983) are inconsistent with previous findings (Sabbadin, 1982). It remains unknown whether gametic and somatic compatibility are functionally correlated in other clonal taxa.

Although very little is known of pathogenic defensive mechanisms of invertebrates, the "host-evasion/pathogen-detection" hypothesis provides a frequency-dependent means for the maintenance of allotypic variation in mammalian, plant, and bacterial defense systems (Bodmer and Bodmer, 1978; Levin, 1986; reviewed in Seger and Hamilton, 1988). This hypothesis rests on the simple assumption that, unless the entire genome is used as a standard of comparison of self with nonself, any self/nonself discrimination system is imperfect. Some allogeneic, or xenogeneic, individuals will simply not be detectable as nonself. Levin (1986) makes a similar argument with his "immunological-window" hypothesis for the maintenance of variation in bacterial restriction-modification systems. Clarke (1979: 464) summarizes the idea, "A parasite in an immunologically competent host could protect itself from attack by reducing its antigenic disparity with the host, and 'mimicking' the host's antigens. This would produce selection in favour of antigenically variant hosts that could damage the parasite."

The "host-evasion/pathogen-detection" hypothesis supposes that selection should act on pathogens to minimize disparity between their haplotypic markers and those of their hosts. In contrast, selection should act on hosts to maximize disparity between a host's and pathogen's haplotypic markers so that the pathogen may be immunologically detectable. Thus, pathogens that are antigenically undetectable should be at a selective advantage relative to those that are detectable as nonself. The magnitude of this selective advantage to a pathogen will depend on the frequency of invadable hosts. From the host's perspective, a host that bears self markers that are rare is unlikely to be matched by a pathogen, because (1) such a pathogen has not evolved or (2) the rarity of such a host confers little selective advantage to an antigenically similar pathogen. Once the host's haplotype increases in frequency, selection should act to increase the frequency of any pathogen which is antigenically similar to the host (hence, can evade immunological detection). Eventually (i.e., at equilibrium), the pathogenic load on the once-rare host haplotype will decrease its selective advantage so that it no longer increases in numbers; selection will then favor a host-haplotype that is novel and not mimicked by any common pathogen. In this way, the fitness of a host haplotype will be inversely proportional to the frequency of pathogens that carry a matching haplotype, and the fitness of a pathogen's haplotype will be directly proportional to the frequency of matching host haplotypes. In other words, selection to avoid pathogens should favor rare host haplotypes, and the strength of selection will depend on the frequency of pathogens capable of evading detection—the more there are, the greater the intensity of selection favoring the rare host genotype that can detect the most common pathogens. Selection is thus frequency-dependent on both host and pathogen haplotypes, and will favor host haplotypes

that are distinct from the haplotypes of pathogens. A number of theoretical analyses (reviewed in Seger and Hamilton, 1988) indicate that such frequency-dependent selection will operate strongly, and polymorphism will be most likely to accumulate, when pathogenic generation times are short relative to those of the host.

CONCLUSIONS

In the context of natural allogeneic interactions between sedentary, clonal invertebrates, theoretical analysis suggests that the costs of allogeneic fusion may be a potent means by which frequency-dependent selection can maintain allotypic variation. It is less clear, however, how the costs and benefits of aggressive behavior can directly or indirectly maintain allotypic specificity.

Pleiotropy can account for the maintenance of allotypic variation, but does not explain why agonistic behavior in cnidarians is so often conditioned on allotype. For such an association to evolve, there must be some benefit to allotypically conditioned aggression. Yet, Grosberg and Quinn (unpub.) have shown that aggressive behavior conditioned on allotypic matching is not evolutionarily stable in the face of either an unconditionally aggressive or an unconditionally passive phenotype. If spatial structure is added to the model such that interactions are strongly biased toward matching allotypes, there is no qualitative change in the outcomes (Grosberg and Quinn, unpub.). Nonetheless, the commonly observed pattern of spatial association of similar allotypes suggests that a complete theoretical analysis should incorporate selection acting at the level of kin groups or demes, and not be limited to the level of the ramet or genet.

Buss and Green (1985) proposed that the existence of allorecognition and allotypic specificity in organisms that generally do not contact allogeneic individuals represents an evolutionary relict from their clonal ancestors. This may be correct; for the reasons discussed in this paper, however, it is difficult to see how the polymorphism required to confer specificity could be maintained in the absence of selection. Yet, even in the absence of allogeneic interactions, the manifold pleiotropic effects of allotypic variation confound any simple analysis of the mechanisms maintaining allospecificity. For example, in other taxa, products of allorecognition loci are thought to regulate (1) immunological responses and the intensity of such responses (McDevitt and Benacerraf, 1969; Klein, 1982; Matzinger and Zamoyska, 1982); (2) tissue differentiation (Bodmer, 1972); (3) patterns of mate-choice (Boyse, Beauchamp, and Yamazaki, 1983; reviewed in Jones and Partridge, 1983) and gametic compatibility (Mattiuz et al., 1970; Oka, 1970; Esser and Blaich, 1973; Scofield et al., 1982); and (4) kin recognition and the distribution of altruistic acts among members of a population (Beecher, 1982; Lacy and Sherman, 1983; Buss, McFadden, and Keene, 1984). All of these mechanisms may play important, but as yet unspecified, roles in controlling levels of allotypic variation in sessile clonal invertebrates.

Some form of frequency-dependent or variable selection appears to be the most likely explanation for the maintenance of allotypic variation. However, until more is known about the genetic architecture of natural populations, the fitness consequences of allogeneic interactions, and the nature and magnitude of the phenotypic effects regulated by allorecognition loci, any general theoretical and mechanistic explanation for the maintenance of allotypic variation and individuality must remain elusive.

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