

Scents of self: The expression component of self/non-self recognition systems

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The ability to distinguish self from non-self is one of the fundamental organizing principles of life on Earth. Such recognition systems permit the unification of distinct elements into cohesive social groups, from multicellular organisms to colonial “superorganisms”. Although the phenomenon of self/non-self recognition has been studied extensively, recent advances have provided fascinating glimpses into the inner workings of recognition systems, and have illuminated some of the extreme forms of selection that drive the evolution of label polymorphism. This polymorphism permits different classes of individuals to be distinguished with high precision, thus creating barriers to outsiders who seek entry into the social group. Here I review the structure and evolution of self/non-self recognition systems across a variety of taxa, with particular attention to the selective forces that maintain label polymorphism. I highlight recent findings that have opened up new avenues of research, and suggest future directions that may provide additional novel insights.

Introduction

The history of life on Earth has been punctuated by several major evolutionary transitions, each of which released organisms from previous constraints, and allowed life to prosper and diversify (Maynard Smith & Szathmáry 1995). Examples include the formation of chromosomes from independent replicators, the endosymbiotic origin of organelles, the formation of the first multicellular organisms, and the aggregation of individuals into cohesive social groups. Although seldom recognized, recognition systems played an important role in many of these innovations. During each transition, the ability of individual subunits to recognize

and exclude dissimilar individuals must have been repressed, thus allowing independent units to combine together and form a new, distinct entity (Buss 1987, Maynard Smith & Szathmáry 1995, Queller & Strassmann 1998, Queller 2000). However, once the transition was completed, a highly precise recognition system was required to prevent random fusion, parasitism by others, or dissolution of the new entity into its component parts. Although great progress has recently been made toward defining the structure and function of many recognition systems, from vertebrate immunity to nestmate recognition in eusocial insects, we have only begun to understand how these systems are modified and maintained by evolutionary forces.

All self/non-self recognition systems must be precise (Grosberg 1988). This precision is commonly achieved through the maintenance of significant polymorphism in the phenotypic traits used for recognition. These traits, or *labels*, are the most conspicuous and well-studied elements of recognition systems. Here, I briefly review the basic structure of recognition systems with particular attention to the role of phenotypic labels. I consider the various evolutionary forces that shape label polymorphism, with a focus on recent examples from the literature. Finally, I identify avenues of future research that are likely to be particularly informative and interesting.

The structure and components of recognition systems

When individuals encounter one another, several components of the recognition system interact to produce the appropriate response (reviewed in Crozier & Dix 1979, Breed & Bennett 1987, Crozier 1987, Grosberg 1988, Reeve 1989, Sherman *et al.* 1997). One individual, the *actor* (or *evaluator*), assesses a phenotypic cue (or *label*) that is present on another individual (the *recipient* or *cue-bearer*). A variety of phenotypes can act as labels, including chemical odors, cell surface proteins, songs, color patterns, or stereotypic displays. The actor compares the recipient's label to a specific set of labels that define "self". This definition, called the *template*, may be genetically hard-wired in the actor or may be acquired during development through processes such as learning or imprinting. When templates are not hard-wired, the reference that they are based upon is known as a *referent*. When the recipient's labels match the actor's template, the actor accepts the recipient as self (Holmes & Sherman 1983, Lacy & Sherman 1983). When the recipient's labels do not match the actor's template, rejection occurs. Different types of recognition systems may employ different matching rules; in some cases, an exact match is required for acceptance, whereas in other systems partial matching is sufficient.

These elements of recognition (labels, templates, referents, and decision rules) can be categorized into three general components, the

expression (or *production*) component, the *perception* component, and the *action* component. The expression component includes the processes involved in the production and expression of labels. This includes the labels themselves, as well as the genes that code for them or modify them. The perception component includes the processes involved in the detection of the recipient's label by the actor, and the comparison of the recipient's label to the actor's template (Mateo 2004). Finally, the action component includes the response (often behavioral) to the label:template match or mismatch (Liebert & Starks 2004). Here I focus on the structure and evolution of the expression component.

Recognition systems are studied in a diverse array of scientific disciplines, which has led to a rich vocabulary of terms, some of which refer to similar or identical processes (*see* Liebert & Starks 2004). For simplicity, I refer to the group of individuals to which the actor belongs as a "colony", a "social group", or "self". These social groups include well-known examples, such as colonies of individual organisms, as well as less commonly considered examples, such as the cells that comprise an individual multicellular organism. Similarly, I refer to recipients that are rejected by the actor as non-self as "non-self individuals", "foreign individuals", or "non-members".

Properties of labels

A common feature of labels is that they are shared by members of the social group, and are not shared by members of different groups. The reasons for this are obvious. When the members of a group possess different labels, they risk rejecting each other as non-members (an error of inappropriate rejection). When this type of rejection occurs in social groups, the group incurs the cost of producing and maintaining the rejected individual, and potentially additional costs associated with the aggressive rejection of that individual (e.g. physical damage or mortality). In species-specific mate recognition, this type of inappropriate rejection results in a missed opportunity for mating or fertilization between compatible individuals or gametes (*see*

Lewis *et al.* 2004). Similarly, in immune recognition systems, inappropriate rejection of self cells can lead to autoimmune disorders, such as lupus, rheumatoid arthritis, and multiple sclerosis (Hood *et al.* 1984, Benjamini 1996). In many cases, members of a group possess the same labels due to kinship within the group. Self/non-self recognition in this context is kin recognition, and acceptance and rejection behaviors allow the actor to preferentially display altruism toward kin, and direct competitive or agonistic behavior away from kin. Thus, indirect fitness benefits may be gained through recognition behavior by members of the group.

When members of different groups possess the same labels, there is a risk of inappropriate acceptance of non-members. This type of recognition error underlies the strategy of many social parasites that evade detection by their host, and are able to infiltrate host colonies. Once inside, these parasites can reduce the fitness of infiltrated colonies by consuming the host's resources or by consuming the hosts themselves. In immune recognition systems, inappropriate acceptance leads to a failure to mount a defensive response to pathogens, parasites, or foreign material (Hood *et al.* 1984, Benjamini 1996).

Both types of recognition errors exert strong selection on recognition systems to be precise. Typically, the precision of recognition is expected to increase with increasing label polymorphism (Grosberg 1988). This is because labels that are common in a population will be shared, due to chance alone, among many individuals. In contrast, when different individuals all share the same rare label, there is a high probability that the label is shared due to common ancestry (in the case of genetically-based recognition) or residence in the same environment, such as a shared nesting site (in the case of environmentally-based recognition).

It is important to note that, in some recognition systems, a template may be shared among members. When this occurs, it is not necessary for group members to possess the same labels. For example, in polygyne ant colonies (which possess multiple reproductive queens) some workers may be distantly related to each other, and therefore labels can vary across individuals (i.e. 'individualistic' recognition, Crozier & Dix

1979). In these colonies, young, newly-eclosed workers can form their template by imprinting on odors that are present in the colony, which include the labels of both relatives and non-relatives. When the workers mature, the "memory" of these odors can then be used as reference against which the labels of others individuals are compared.

Self/non-self recognition in marine invertebrates

For sessile marine invertebrates, physical space is often a limiting resource. Consequently, as colonies grow they may come into contact with colonies of different species as well as foreign colonies of the same species. In many cases, the nature and consequences of these interactions are controlled by self/non-self recognition systems in which high levels of label diversity facilitate precision in recognition (reviewed in Hildemann *et al.* 1979, Grosberg 1988). In fact, it is clear that such recognition systems are present in a wide range of marine invertebrates, including sponges, most cnidaria, and tunicates (Hildemann *et al.* 1979).

Early studies of the colonial ascidian, *Botryllus schlosseri*, revealed that encounters between different species produce markedly different reactions than encounters between two *Botryllus* colonies (Bancroft 1903, Oka & Watanabe 1957, Oka 1970). When two heterospecific colonies meet, both typically continue to grow with no discernable allorejection — each essentially treating the other as substrate. Contacts between two *Botryllus* colonies, on the other hand, frequently lead to an active inflammatory reaction, rejection, and the formation of a necrotic barrier between the two colonies (Burnet 1971). However, this aggressive rejection is not displayed toward all *Botryllus* colonies. When close kin come into contact, or when separate clones are propagated from the same initial colony and are subsequently permitted to reestablish contact, the two may fuse together, forming a continuous gastrovascular system.

Genetic crosses have revealed, as originally proposed by Oka and Watanabe (1957), that the labels used in the self/non-self recognition system

of *Botryllus* are controlled by a single, highly polymorphic locus, the fusion/histocompatibility locus (Fu/HC) (Scofield *et al.* 1982). *Botryllus* colonies fuse when they share one or both alleles at this locus; rejection occurs when no alleles are shared. As in other recognition systems, label diversity at the Fu/HC locus is extremely high. For example, Grosberg and Quinn (1986) reported a fusion frequency consistent with the presence of approximately 100 alleles within a single population in Woods Hole, MA. Similarly, Rickevich *et al.* (1995) used allerejection assays to show that the number of alleles in Mediterranean populations of *B. schlosseri* ranged from 58 to 306 within sampling localities on the order of 1400–5000 m².

As in *Botryllus*, encounters between distantly related individuals lead to rejection in anthozoan and hydrozoan cnidaria (Grosberg 1988, Ayre & Grosberg 1995, 1996, Grosberg *et al.* 1996, Hart & Grosberg 1999), often in concert with highly damaging battles that involve specialized fighting structures (Buss *et al.* 1984, Williams 1991). One well-studied example is the colonial hydrozoan, *Hydractinia symbiolongicarpus*, which colonizes gastropod shells that are occupied by the hermit crab, *Pagurus longicarpus* (Buss & Yund 1989). Occasionally, multiple larvae colonize the same shell, metamorphose, and grow into contact with each other (Yund *et al.* 1987, Yund & Parker 1989). These encounters between *Hydractinia* colonies lead to one of three potential outcomes: aggressive rejection, fusion, or transitory fusion. When rejection occurs, the colonies typically attack each other with specialized fighting structures, called hyperplastic stolons, which are deployed from the gastrovascular system and contain large numbers of nematocytes (Ivker 1972). At the opposite extreme, when fusion occurs, two colonies join together into a single colony that possesses a shared gastrovascular system. Such fusion increases overall colony size, which is known to increase survivorship and decrease mortality in a variety of marine organisms (e.g. Buss 1980, Highsmith *et al.* 1980, Hughes & Jackson 1985). Transitory fusion is characterized by an initial fusion between two colonies, followed by unilateral or bilateral rejection (Shenk & Buss 1991).

The genetics of allerecognition appears to be substantially more complex in *Hydractinia* than in *Botryllus*. Crossing experiments have shown that about 30% of full sibs are compatible, whereas only about 2% half-sibs are (Grosberg *et al.* 1996). These fusion frequencies are consistent with a recognition system that consists of about 5 loci, each possessing approximately 5–7 alleles (Grosberg *et al.* 1996). Genetic analysis of populations in the field have shown that related larvae frequently colonize the same shell, indicating that there are abundant opportunities for fusion to occur (Hart & Grosberg 1999).

A number of different costs and benefits may promote recognition specificity by selecting for heightened label polymorphism. In marine invertebrates, some of the potential benefits of fusion are clear. Because environmental forces (such as erosion or substrate movement in the intertidal) can cause colony subdivision or fragmentation, a failure to subsequently recognize and fuse with self fragments could lead to direct competition between identical clones. Also, encrusting colonies often envelop their substrate, which requires a mechanism for recognition to occur between opposite sides of a single colony, permitting them to fuse together. Although it has been suggested that mate selection may drive the high level of label polymorphism (Oka 1970, Scofield *et al.* 1982), crossing studies have shown that this is unlikely. Grosberg and Hart (2000) performed crosses between fusion-compatible and incompatible colonies of both *Botryllus schlosseri* and *Hydractinia symbiolongicarpus*. Analysis of the resulting progeny showed that crosses between colonies that were fusion-compatible did not yield fewer offspring than crosses between incompatible parents, and crosses between semi-compatible colonies did not produce progeny classes with significantly fewer homozygotes than heterozygotes (Grosberg & Hart 2000).

Recent findings suggest that post-settlement differences in viability between homozygotes and heterozygotes at the Fu/HC locus may promote label diversity. When the survival of progeny from different types of crosses was tracked for the first ten weeks of *Botryllus* development, heterozygotes at Fu/HC had a significantly higher probability of surviving to sexual maturity (De Tomaso & Weissman 2004). This type of

developmentally regulated overdominance (heterozygote advantage) may prove to be a powerful force driving label polymorphism in this and other self/non-self recognition systems.

Intraspecific parasitism also appears to be an important force driving recognition specificity in colonial marine invertebrates. These species, like most organisms (and unlike the higher metazoa with which we are most familiar), do not sequester their germ lines early in the developmental process (Buss 1987). Instead, stem cells or primordial germ cells remain undifferentiated within the organism until sexual maturity. For organisms that are able to fuse with conspecifics, the presence of these potential reproductive cells poses a very serious problem: How does one prevent parasitism by fusion partners who can inject their reproductive cells into your soma?

Several studies have shown that such parasitism occurs in some taxa (Strassmann *et al.* 2000, Velicer *et al.* 2000), including *Botryllus schlosseri* (Pancer *et al.* 1994, Stoner & Weissman 1996, Stoner *et al.* 1999). Early studies of post-fusion *Botryllus* chimeras observed that germ line cells were exchanged between the fused individuals and, in many cases, one individual in the pair contributed cells that appeared to be more successful (Sabbadin & Zaniolo 1979). Later work using microsatellite markers confirmed this finding (Pancer *et al.* 1994, Stoner & Weissman 1996) and showed that fusion and subsequent germ cell transfer occurs within chimeras in the field (Stoner & Weissman 1996). The ability of some clones to act as successful parasites relative to other clones is a heritable trait (Stoner *et al.* 1999), indicating that the evolution of highly polymorphic self/non-self recognition systems has likely been driven, in part, by the need to exclude parasitic cell lineages by limiting fusion to close kin (Buss 1982, Grosberg & Quinn 1986, Buss 1987, Grosberg 1988, Stoner & Weissman 1996, Buss 1999, Stoner *et al.* 1999).

Self/non-self recognition in social insects

Although the ability of eusocial insects to distinguish nestmates from non-nestmates has been

recognized for over a century (Lubbock 1882, Wheeler 1900, Fielde 1903b, 1903a, 1904), the development of kin selection theory in the 1960s (Hamilton 1964) renewed interest in the evolution and structure of social insect recognition systems. In eusocial species, the fitness of sterile individuals hinges upon their ability to direct altruistic behaviors toward kin (reviewed in Hölldobler & Wilson 1990, Bourke & Franks 1995, Crozier & Pamilo 1996). This produces strong selection for the development of mechanisms that permit kin to be distinguished from unrelated individuals. In many cases, social insect colonies are family groups, which allows colony-mate (or nestmate) recognition to evolve as a proxy for kin recognition. Although kin selection has produced a rich theoretical foundation describing the evolution of social behavior and associated costs and benefits of altruism, direct exploration of the expression component and identification of specific labels that are used for recognition have proven somewhat elusive. The primary complicating factors in this area are the complexity of social insect recognition systems, in terms of the nature of the labels used for recognition, and the variation and plasticity in other components of recognition, which can obscure expression-related processes. Moreover, the demographic structure of colonies and the relative contribution of workers and queens to the colony odor vary substantially across taxa, and occasionally within species.

In the eusocial Hymenoptera, it is clear that chemical odor cues are the most common type of label used for recognition. In many cases, cuticular hydrocarbons have been implicated as the general type of compound that forms the colony-specific odor (reviewed in Gamboa *et al.* 1986b, Breed & Bennett 1987, Breed 1998, Singer 1998). These hydrocarbons are waxy substances on the exoskeleton of insects that likely evolved to aid in desiccation resistance, and only later were co-opted for recognition (Blomquist *et al.* 1998). Because cuticular hydrocarbons are not direct gene products (proteins), the genetic underpinnings of hydrocarbon polymorphism remain largely unknown.

In ants, the labels for recognition can be produced by workers (e.g. Haskins & Haskins 1979, Mintzer 1982, Stuart 1988, Bennett 1989,

Stuart 1991), queens (e.g. Haskins & Haskins 1950, Hölldobler & Taylor 1983), or both (e.g. Crozier & Dix 1979, Carlin & Hölldobler 1983, Carlin & Hölldobler 1986, Crosland 1989, Stuart & Herbers 2000). These chemicals may then be distributed throughout the membership of the colony via allogrooming and trophallaxis, resulting in a colony-specific odor blend, or “gestalt” (Crozier & Dix 1979).

Ants in the genus *Temnothorax* (formerly *Leptothorax*) are particularly well-studied. Experimental work by Stuart and colleagues dissected the contribution of various colony attributes to recognition in *T. ambiguous* and *T. longispinosus* (Stuart 1988, Stuart & Herbers 2000). When pupae were removed from colonies, permitted to eclose in isolation, and reintroduced into their natal colonies, they were rarely attacked by their nestmates (Stuart 1988). In contrast, when workers that eclosed in isolation were introduced to foreign colonies, they were rejected significantly more often (Stuart 1988). These data indicate that recognition labels in these species are probably genetically encoded. When pupae were cross-fostered in heterospecific colonies and reintroduced into their natal colonies, they were typically rejected by their conspecific nestmates (provided they were not reared with large numbers of conspecific fosterlings, Stuart 1988). These data indicate that the recognition labels are transferred, although not completely (Stuart 1988), among individual workers. GC/MS analysis of individual *T. acervorum* and *T. gredleri* workers has shown that cuticular hydrocarbons differ among colonies, as expected for labels that are used in nestmate recognition (Tentschert *et al.* 2002).

A variety of studies have shown that colony members can produce substances that are incorporated into the nesting material (reviewed in Gamboa *et al.* 1986a, Breed 1998). Some of the most convincing studies of this phenomenon have been performed in the European honey bee, *Apis mellifera* (reviewed in Breed 1998). Comparisons of honey bee comb wax across different families showed that substantial hydrocarbon variation exists, indicating that they could be used effectively as labels (Page *et al.* 1991, Breed *et al.* 1995b). Additionally, studies in which bees were exposed to wax from different

colonies provided further evidence that comb wax hydrocarbons are involved in nestmate recognition (Breed *et al.* 1995a, Breed *et al.* 1995b). Newly emerged bees that were exposed to comb wax for a short period of time (as little as 5 minutes) were rejected in only 32% of trials by bees that had been exposed to either the same comb or comb from a closely related colony. In contrast, when newly emerged bees were exposed to comb wax, then presented to groups that were exposed to comb from unrelated colonies, the rejection rate was significantly higher (about 73%). Manipulative experiments in which compounds were extracted from wax and tested for activity in recognition assays have permitted the identification of numerous potential labels (Breed & Stiller 1992, Breed 1998). These studies have shown that, in general, alkenes are more likely than alkanes to produce responses consistent with their use as recognition labels (reviewed in Breed 1998).

Wasps, like ants and bees, use chemical odor cues as labels for recognition (reviewed in Gamboa *et al.* 1986a, Singer 1998, Gamboa 2004), and young wasps form a template by learning or imprinting on these odors soon after eclosion (Shellman & Gamboa 1982, Pfennig *et al.* 1983a, Pfennig *et al.* 1983b). For example, when newly emerged workers spend the first few days after eclosion on nests that have been cleansed of hydrocarbons, they are unable to subsequently distinguish nestmates from non-nestmates (Singer & Espelie 1992). Wasps reared on control, untreated nests displayed normal recognition behavior (Singer & Espelie 1992).

Several studies have sought to identify specific hydrocarbons that are used for recognition by wasps. When the behavior of *Polistes fuscatus* was compared between colonies reared in the field and in the laboratory, Gamboa *et al.* (1986a) found that field-reared *P. fuscatus* displayed more stringent recognition behavior, pointing to a role for environmentally-derived labels. However, individuals reared in the laboratory were still able to distinguish nestmates from non-nestmates, indicating that heritable cues are also important. Similarly, correlative studies in *P. fuscatus* have provided strong evidence that a handful of particular hydrocarbons, prima-

rily methyl-branched alkanes and straight-chain alkanes, are most likely the labels used (Espelie *et al.* 1994, Gamboa *et al.* 1996).

More recent manipulations of the hydrocarbon profiles of individual wasps have provided additional convincing evidence that hydrocarbons are used for recognition, and have permitted the identification of active compounds in two species, *Vespa crabro* (Ruther *et al.* 2002) and *P. dominulus* (Dani *et al.* 2001). Ruther *et al.* (2002) extracted hydrocarbons from *V. crabro* workers, applied them to dead, washed workers, and introduced the treated bodies back into their colony. When dead workers were treated with hydrocarbons from nestmates, no aggression was observed. However, when the nestmate hydrocarbons were spiked with a small amount of three different candidate hydrocarbons, aggression toward the treated bodies increased significantly. In a similar experiment, Dani *et al.* (2001) synthesized linear alkanes, alkenes, and methyl-branched alkanes that are known to occur on the exoskeleton of *P. dominulus* wasps. When individual (living) wasps were treated with these compounds and reintroduced into their nest, those that had been treated with linear alkanes did not elicit a detectable response. In contrast, application of methyl-branched alkanes or alkenes resulted in aggression toward treated individuals by their nestmates.

In a fascinating study of *P. sulcifer*, a social parasite of *P. dominulus*, Sledge and colleagues (2001) illustrated one of the potential vulnerabilities of social insect recognition systems, and were able to identify candidate labels using a unique approach. *Polistes sulcifer* is an obligate social parasite, and does not produce workers. During the late spring, mated *P. sulcifer* females emerge from hibernation and seek out colonies of *P. dominulus*, which are founded a few weeks prior to *P. sulcifer* emergence. After locating a host colony, the *P. sulcifer* female enters and eliminates the *P. dominulus* foundress by killing her or driving her from the colony. The parasite then begins producing offspring, which are reared by remaining *P. dominulus* workers. Several lines of evidence indicate that the acceptance of *P. sulcifer* by the host workers is mediated by a clever chemical disguise. Sledge *et al.* (2001) first removed parasites from host colonies

and presented them to *P. dominulus* workers from either their own parasitized colony, or to a foreign *P. dominulus* colony that was parasitized by a different *P. sulcifer* individual. Hosts from the parasite's own colony displayed virtually no aggression toward her, but hosts from foreign parasitized colonies aggressively rejected *P. sulcifer* from different host colonies. Next, *P. sulcifer* females were removed from colonies, frozen, and washed with hexane to extract cuticular hydrocarbons. These hydrocarbons were then applied to *P. dominulus* foundresses that had been frozen and subsequently cleansed of cuticular compounds. These *P. dominulus* bodies + *P. sulcifer* hydrocarbons were then introduced, as before, to either the host colony of origin or to a foreign parasitized host colony. Again, *P. dominulus* workers accepted the bodies that possessed hydrocarbons from their own *P. sulcifer*, but aggressively rejected bodies treated with hydrocarbons of *P. sulcifer* that parasitized foreign *P. dominulus* colonies. This demonstrated that hydrocarbon extracts from *P. sulcifer* contain the colony-specific labels that facilitate acceptance. Finally, an analysis of both host and parasite hydrocarbons using gas chromatography-mass spectroscopy (GC-MS) showed that, subsequent to usurping the host colony, *P. sulcifer* individuals acquired hydrocarbon profiles that matched the distinctive profile of their host colony, a finding that matches the results of previous studies (Turillazzi *et al.* 2000). Although it has not yet been determined if *P. sulcifer* parasites are mimicking their host's hydrocarbon profile, or if the parasites are simply acquiring labels synthesized by the host, the close, colony-specific match between host and parasite suggests that the latter is more likely.

Colony-specific odors may also be acquired by social insects from environmental sources, such as food items, nesting materials, or substrate. In theory, recognition systems based on environmentally-derived sources can be used to effectively distinguish colony members from non-members when the environmental odor varies spatially, or is sufficiently polymorphic to permit different, neighboring colonies to acquire detectable olfactory differences. However, when non-heritable cues are used as a basis for acceptance and rejection, these behaviors can easily

become decoupled from kinship. When this occurs, altruistic behaviors may be displayed toward unrelated individuals, thereby eliminating the inclusive fitness benefits of recognition. Additionally, when environmentally-derived labels are used for recognition, non-colony members that can acquire them from the source may then enter the colony unmolested, and wreak havoc within.

Evidence for the use of environmentally-derived labels is mixed. In the honey bee, for example, there are data that both support (Ribbands *et al.* 1952) and refute (Bowden *et al.* 1998, Downs *et al.* 2001) the proposal that floral oils are used for colony-specific recognition. In ants, environmentally-acquired odors appear to be used less frequently than genetically-encoded cues (reviewed in Ribbands 1965, Breed & Bennett 1987). In many cases, however, differences in environmental odors may act synergistically with genetic differences between colonies. For example, studies of the leaf-cutter ant *Acromyrmex octospinosus* showed that when colonies were divided and provided with different types of forage, they displayed non-injurious aggression when combined back together (Jutsum 1979). When different colonies were provided with the same type of forage, they displayed lower levels of aggression toward each other than colonies that were provided with different forage (Jutsum 1979). Similarly, studies of six species of *Camponotus* showed that queenless colonies used both environmental and worker-derived cues for recognition, although the latter appeared to take precedence over the former (Carlin & Hölldobler 1986).

Termites may possess a novel source of distinctive colony labels — the fauna of their own digestive tracts. Experiments using the termite *Reticulotermes speratus* have suggested that altering the gut bacteria affects patterns of nestmate recognition (Matsuura 2001). Matsuura (2001) extracted and cultured bacteria from the guts of individual workers, then applied these bacteria to sawdust baits. A subset of *R. speratus* workers were then fed baits containing bacteria from foreign termite colonies, and reintroduced into their natal colonies. When compared with negative controls (termites fed baits that had been treated with distilled water, but no bacte-

ria) treatment workers survived significantly less often, presumably as a consequence of aggressive rejection by their natal colony. Interestingly, when replicate colonies were fed different types of antibiotics and subsequently tested against each other, colonies that had been fed the same type of antibiotic displayed lower levels of aggression than colonies that had been fed different types of antibiotic. This finding suggests that altering the bacterial content of the gut not only alters the labels used for recognition, but also produces correlated changes in the termites' template. Not only did behavior toward antibiotic-treated termites change, but the behaviors displayed by them changed as well. These preliminary findings highlight the need for further exploration of the role that symbionts may play in nestmate recognition.

Although chemical communication is common among social insects, some social insects also utilize different sensory modalities for recognition. Tibbetts (2002) recently showed that individual facial patterns and abdominal markings are highly polymorphic within colonies of the paper wasp, *Polistes fuscatus*, suggesting an opportunity for them to be used as labels for individual identification. When these patterns were altered by either adding new markings or obscuring existing markings with black paint, levels of aggression displayed by nestmates toward the treated individuals increased. In contrast, aggression toward control individuals, which were painted without altering the facial or abdominal markings, did not change. These results are consistent with a visually-based system of individual recognition, which likely acts to maintain the dominance hierarchy within *Polistes* colonies. With luck, future genetic studies will reveal the degree to which these visual labels are heritable, and will elucidate the selective forces that act upon them.

Self-incompatibility in plants

The effects of inbreeding depression have been documented in a variety of plants by many different researchers (Darwin 1876, Wright 1977, Charlesworth & Charlesworth 1987). In response to the deleterious effects associated with selfing,

many plants have evolved precise systems for recognizing and rejecting their own pollen and the pollen of close relatives. There are two basic types of single-locus self-incompatibility: sporophytic self-incompatibility (SSI) and gametophytic self-incompatibility (GSI) (reviewed in de Nettancourt 1977, Richman & Kohn 1996, Richman 2000). In species with SSI, the haploid pollen grain expresses both parental S-alleles, and is rejected by flowers of plants that possess either of these two alleles. In GSI systems, individual plants produce haploid pollen that expresses one of the two parental alleles at the S-locus. If this pollen lands on the stigma of a plant that possesses the same allele, a complete pollen tube fails to grow and fertilization does not occur. Thus, SSI represents a more stringent form of self/non-self recognition; species with SSI reject all pollen from plants that share a single S-allele whereas species with GSI accept half of the pollen from plants that share a single S-allele.

These systems of self-incompatibility produce strong negative frequency dependent selection (or balancing selection) on the S-locus (reviewed in de Nettancourt 1977, Richman & Kohn 1996, Richman 2000). Plants that possess rare alleles produce pollen that can pollinate many different plants, whereas plants with common S-alleles are able to pollinate few plants. Thus, the frequency of a plant's mating success is inversely proportional to the frequency of its S-alleles in the population. Under this negative frequency-dependent selection, the frequency of new S-alleles increases rapidly to $1/k$, where k = the total number of different S-alleles in the population. Individual S-alleles can also persist for extremely long time spans because the loss of rare alleles through genetic drift is ameliorated by the negative frequency-dependent selection (Vekemans & Slatkin 1994).

In accordance with theoretical expectations, natural populations of self-incompatible plants possess high levels of polymorphism at the S-locus (Richman & Kohn 1996). For example, Emerson (1939) reported that 45 different S-alleles were present in a single, relatively small population of *Oenothera organensis*. Similarly, Richman and colleagues (Richman *et al.* 1996) used reverse transcription-PCR (RT-PCR) to

directly examine the diversity of S-alleles in *Physalis crassifolia*, a solanaceous desert perennial. They found 28 alleles in the first 22 individuals screened, which translated to an estimated 44 alleles within this one population.

Finally, it is worth noting that systems of plant self-incompatibility bear remarkable similarities to other genetic systems, such as Hymenopteran sex determination (Bull 1983, Charlesworth 2003) and some fungal mating systems (Caselton 2002), in which large numbers of alleles are maintained by selection. In these systems, as in plant self-incompatibility, the viability or fertility of individuals depends on the presence of different alleles at a single locus. The resulting negative frequency-dependent selection drives the proliferation of allelic diversity at these loci, and the maintenance of novel alleles over long evolutionary time spans.

Crozier's paradox and frequency-dependent selection against label diversity

Although label polymorphism is essential for effective recognition, under some circumstances selection is expected to purge label diversity from recognition systems. This occurs when there are fitness costs associated with rejection (Crozier 1986, 1987, 1988, Elgar & Crozier 1989, Grosberg & Quinn 1989, Tsutsui *et al.* 2003). These costs can include physical damage associated with agonistic encounters, the expense of producing specialized fighting apparatus, or an increased likelihood of death.

The uneven distribution of these costs poses a serious problem for the maintenance of label diversity (Crozier 1986, 1987, 1988, Elgar & Crozier 1989, Grosberg & Quinn 1989, Tsutsui *et al.* 2003). Individuals that possess rare labels have a high probability of being rejected, and incurring the associated costs, because rare labels translate into a low probability of matching an actor's template. Conversely, because common labels match many templates, individuals that possess them are seldom subjected to the costs of rejection. Thus, through time, selection should purge rare labels from the population, decreasing label diversity. Because most recognition

systems rely upon high levels of label polymorphism to function correctly, this selection poses a paradox, first described and developed by Crozier (Crozier 1986, 1987, 1988, Elgar & Crozier 1989). That is, how can recognition systems maintain the necessary level of label polymorphism when the act of rejecting non-members decreases the same polymorphism?

Although few empirical studies have focused on this problem, work on the invasive Argentine ant (*Linepithema humile*) has provided evidence supporting Crozier's paradox. In the early 1900's, researchers noted that introduced populations of Argentine ants displayed no intraspecific aggression or territoriality, even across large spatial scales (Newell & Barber 1913). More recent studies of introduced North American populations have shown that this lack of aggression is associated with low levels of genetic variation and increased genetic similarity (Tsutsui *et al.* 2000, 2003, Tsutsui & Suarez 2003). In contrast, Argentine ants in their native South American range display high levels of intraspecific aggression at much smaller spatial scales (Suarez *et al.* 1999, Tsutsui *et al.* 2000, Tsutsui & Case 2001). Although the introduced range in California is dominated by a single large supercolony, smaller "secondary" colonies are also present (Tsutsui *et al.* 2000, 2003, Tsutsui & Case 2001). These secondary colonies likely arose from additional, independent introductions of Argentine ants from the native range, or from elsewhere in the introduced range (Tsutsui *et al.* 2001).

Behavioral and genetic studies show that Argentine ants, like many other eusocial insects, can use cues of genetic similarity (probably cuticular hydrocarbons, Suarez *et al.* 2002), to distinguish colony members from nonmembers (Tsutsui *et al.* 2000, Tsutsui & Case 2001). Interestingly, aggression between pairs of colonies in the introduced range is highly polarized, with workers from one colony typically acting as the attackers (Tsutsui *et al.* 2003). Moreover, these colonies form a hierarchy, with some colonies, including the large supercolony that dominates California, almost always acting as attackers, whereas other colonies are typically the recipients of aggression (Tsutsui *et al.* 2003). Genetic analysis using microsatellite markers

reveals a pattern consistent with Crozier's predictions: the most aggressive colonies possess common alleles whereas the recipient colonies typically possess higher levels of diversity, and a larger number of rare alleles. When low-diversity colonies are constructed in the lab from a more diverse source colony, the same pattern is observed (Tsutsui *et al.* 2003). Finally, examination of post-aggression mortality shows that individual attackers survive agonistic encounters about six times more often than the recipients of aggression (Tsutsui *et al.* 2003). Thus, in Argentine ant recognition, there is a clear cost associated with being rejected, and ants from high-diversity colonies are rejected more often than ants from low-diversity colonies. Under this type of positive frequency-dependent selection, high-diversity colonies are expected to be at a disadvantage relative to low-diversity colonies, and label diversity should gradually be purged from the population. Currently, however, the colony-level consequences of this asymmetry remain unexplored in the field.

Although other studies have not directly examined the possibility of positive frequency-dependent selection, there are tantalizing hints that this process is widespread. Data from a variety of social insects indicates that individuals from monogyne (or low-diversity) colonies possess more stringent recognition systems than individuals from polygyne colonies (Janzen 1973, Breed & Bennett 1987, Obin & Vander Meer 1989, Morel *et al.* 1990, Starks *et al.* 1998, Pirk *et al.* 2001). Marked asymmetries in aggression have also been noted among colonies of other species (e.g. Mintzer 1982, Stuart & Herbers 2000). Differences in label diversity may give rise to these asymmetries because (1) ants in low-diversity colonies imprint on a smaller number of labels during template formation and/or (2) ants in high-diversity colonies are marked with rare, frequently-rejected labels. A similar pattern has been observed among colonies of some marine invertebrates. In the sea anemone *Anthopleura elegantissima* there appears to be a positive relationship between dominance rank and homozygosity, and attackers prevail significantly more often than the recipients of aggression (Ayre & Grosberg 1995, Grosberg & Ayre 1997, Zeh & Zeh 1997). Similarly, when fusion

occurs between colonies of *Botryllus schlosseri*, one clone is often resorbed by others (Rinkevich *et al.* 1993). The “winners” of these encounters (the clones that are not resorbed) are consistent across different pairings, forming a hierarchy similar to that observed among Argentine ant colonies (Rinkevich *et al.* 1993). In these examples, the asymmetry in aggression between interacting social groups suggests that the costs and benefits associated with rejection behavior are also distributed unevenly.

Conclusions and future directions

To date, few loci that encode phenotypic labels have been cloned, the two primary exceptions being the plant S-locus and the vertebrate MHC. Ongoing work appears to be closing in on the *Botryllus* Fu/HC locus (De Tomaso & Weissman 2003), and some genes in the enzymatic pathways controlling hydrocarbon synthesis in insects are known (Blomquist *et al.* 1998). As specific labels, and the genes that encode them, are identified for these and other taxa, we will be able to more fully grasp how selective forces have shaped the structure of different recognition systems, and fundamental shared properties will become evident. The general importance of self/non-self recognition across a wide variety of disciplines offers hope that these discoveries will be widely applicable.

In many cases, it is clear that reproductive parasitism is a potentially strong force driving precision and specificity in self/non-self recognition systems. In colonial marine invertebrates this results from the transfer of multipotent stem cells, whereas in eusocial insects the equivalent occurs when a reproductive foreign individual is mistakenly accepted as a nestmate. In both cases, non-reproductive elements of the colony (somatic tissue or sterile workers, respectively) are co-opted for the reproductive benefit of non-kin. At present, we know much more about the frequency of such intraspecific social parasitism, and hence the selective pressure exerted by it, in marine invertebrate systems. However, the experimental tractability of many social insect systems combined with the power of molecular tools offers hope that we will soon see greater

exploration of this phenomenon in a wider array of species (e.g. Kronauer *et al.* 2003).

Similarly, empirical studies are beginning to bear out the predictions of theory regarding the costs of rejection, and the associated frequency-dependent selection that this may impose upon label diversity. Future studies that explicitly test the hypothesis that asymmetrical rejection is biased with respect to genetic diversity will reveal the extent to which this type of behaviorally-mediated selection shapes the expression component of self/non-self recognition systems.

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