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Kin Recognition and Genetics

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Introduction: A Gene's Eye View of Altruism

At first glance, altruistic behaviors – those that benefit others at the actor's expense – are not well suited to the natural world. Within a population, individuals compete for access to food, mates, and habitats, and only those that are best able to secure those resources survive and reproduce. Given this struggle for resources – what Darwin called the 'dreadful, but quiet war of organic beings' – it is reasonable to assume that selfish and exploitive behaviors would always prevail.

This does not, however, appear to be the case. While nature has more than its share of selfishness, it proves surprisingly hospitable to cooperation, sharing, and self-sacrifice. Studies of animal behavior provide numerous examples. When faced with famine, some unicellular slime moulds sacrifice themselves to help others escape. Vervet monkeys put themselves at increased risk to warn others of an approaching predator. Perhaps most spectacularly, the eusocial insects have evolved sterile worker castes, the members of which give up their own reproduction for the sake of their nestmates⁷.

Darwin was particularly impressed with the last example and saw it as a formidable challenge to natural selection: he went so far as to call the evolution of worker ants 'by far the most serious special difficulty which my theory has encountered.' His solution – largely ignored for over a century – was to recast natural selection in terms of competition between families; Darwin reasoned that if a sterile ant worker increased its family's overall fitness, then it might also increase the chances that its own traits would recur in the next generation. In the early 1960s, William Hamilton revisited this idea with mathematical rigor in a series of papers that challenged the way behavioral ecologists thought about altruism. In them, he introduced and formalized the concept of inclusive fitness, a framework for viewing natural selection from the perspective of genes rather than of individuals. Such a

paradigm shift has profound implications for the study of altruism: from a gene's point of view, no behavior is selfless as long as it results in an increased number of copies of that gene in the next generation. According to Hamilton, even if an individual sacrifices its life, the genes responsible for that sacrifice can still spread through the population so long as $rb - c > 0$ (Hamilton's rule, where b is the benefit to the recipient of a behavior, c is the cost incurred by the actor, and r is the proportion of genes that the two share by common descent). Altruism is therefore advantageous only under certain conditions and only when it is preferentially directed toward close relatives, where r is highest and the number of shared genes is greatest.

Forty years on, it is hard to overestimate the impact that inclusive fitness theory has had on the study of animal behavior. Gene-level explanations help us to understand why some wasps forgo reproduction and instead raise sisters, why scrub jays sometimes devote their youths to defending their parents' nests, and why baboons often risk their lives to protect their kin groups. But these explanations also generate new questions and new directions for research. One such direction – research into the existence and nature of kin recognition systems – has proved particularly fruitful. The central question arises directly from Hamilton's insights: If we expect organisms to behave in ways that preferentially benefit their close relatives, should we not also expect them to identify those relatives? If they do discriminate between individuals on the basis of relatedness, what mechanisms might they use to do so? More importantly for our purposes, to what extent are those mechanisms influenced by genetics?

Since the late 1970s, researchers have produced convincing evidence that many animals do in fact discriminate between relatives and nonrelatives. Organisms as diverse as sweat bees, bank swallows, sea squirts, and macaques – even some plants and microorganisms – all demonstrate some form of kin recognition and discrimination when directing care or choosing mates. Biologists

have also come a long way in describing the mechanisms behind this recognition, but much remains unknown about the relative contributions of genetic factors. In this article, we review our current understanding of the genetics of kin recognition systems and describe some of the mechanisms animals use to recognize relatives.

A Recognition Lexicon: Describing the Components of a Recognition System

The terms used to describe recognition and communication systems have fluctuated considerably over the last 25 years: what some authors have called recipients, others have called signalers; and what some call receivers, others call actors. For consistency and clarity, we suggest that the following terms be used to describe recognition behaviors.

All recognition systems, whether they identify kin, evaluate mates, or discover predators, require at least two participants: an agent that gets recognized and another that does the recognizing. We use the terms cue-bearer and evaluator, respectively, to clarify these different roles. Evaluators must also possess some set of criteria against which to compare a cue-bearer and make a decision about its identity. This identification key is called a template, and it can be either fixed at birth or learned later during development. Some of these learned templates are formed early on and quickly become resistant to further modification; others, meanwhile, are subject to constant revision and may change dramatically throughout an organism's lifetime. In either case, the learned template is established with reference to some external model, the so-called referent. Although the referent is most often another individual – usually a nestmate, a parent, or some other relative – it may also be the organism itself, or, as in some social insects, the organism's natal nest.

Researchers have found it useful to divide the recognition process into three essential and discrete parts: the so-called expression, perception, and action components. Expression refers to the production or acquisition of identity cues by the cue-bearer; perception entails the recognition and interpretation of those cues by the evaluator; and action describes all those behaviors elicited on the part of the evaluator by the act of recognition. Organisms demonstrate varying degrees of genetic involvement in each of these components, and in many cases the relative contributions of genes remains unclear.

Finally, it is worth pointing out the fine distinction between kin discrimination and kin recognition. Although the terms are sometimes used interchangeably, the former properly refers to an observed behavioral change in the presence of kin, while the latter refers to processes occurring inside the evaluator. We can easily imagine an

organism that, while fully aware of who is who in its social group, does not modify its behavior in response to that information. Such an instance would resist typical empirical analysis and would belie the presence of recognition. Keeping the terms separate not only helps us design more rigorous analyses but also provides a humbling reminder of what we can and cannot know about animal minds.

The Gull and the Egg-Shaped Rock: Is a Genetic Component Necessary?

Genes for altruism thrive only if they direct help toward other individuals carrying those genes. As such, we might expect successful recognition systems to evaluate genotypes directly by looking for unambiguous phenotypes that reveal matches at specific loci. However, instances of such direct allelic recognition are exceedingly rare; much more common are systems that look not for specific alleles, but rather for indicators of overall relatedness. These 'best guess' mechanisms use those indicators to approximate the degree of kinship and thus the likelihood that any given allele is shared. If, for instance, you can safely assume that another individual is your brother, then there is a 50% chance that you and he will share any given allele; if the individual is only your half-brother, then the chance falls to 25%. Recognition systems that determine approximate kinship can therefore help organisms make appropriate behavioral choices even in the absence of detailed genotypic information. While many of these mechanisms depend on matching newly encountered phenotypes to recognition templates, some do not evaluate phenotypes at all. These are the so-called context-dependent mechanisms, and they are the focus of this section.

Imagine a couple driving home from the grocery store with their infant in the backseat. Knowing that they had strapped their child in just minutes before, they would be surprised – and more than a little nonplussed – to turn around and find a different infant smiling back. As long as certain situations are always associated with kinship (e.g., the backseat of the couple's car normally contains only their child), they may serve as reliable indicators of relatedness. In his 1964 paper, Hamilton cites Niko Tinbergen's example of the herring gull: Through controlled experiments, Tinbergen found that these birds were incapable of differentiating between their own eggs and any egg-shaped objects placed inside their nests. In fact, anything that approximated the correct shape and color was treated with the care due to offspring. 'This is what we would expect,' Hamilton concludes, 'in view of the fact that eggs do not stray at all.' In other words, the gull has little reason to suspect that an egg-shaped object sitting in its nest is anything other than its offspring.

Similar context-dependent recognition is found in some swallows, dunnocks, and prairie dogs, among others.

The bank swallow (*Riparia riparia*) is a particularly useful example, as its kin recognition system shifts to match conditions found at different periods of parental care. Shortly after they hatch, the altricial offspring are confined to natal nests, and parents have good reason for supposing that any young found in those nests are their offspring. At this stage, the swallows operate under a reasonable-assumption, context-dependent recognition rule: if a chick is sitting in your nest, treat it as though it were your own. As the chicks age, however, they leave the nest more frequently and begin to intermingle more freely with other individuals in their age group. This creates more opportunities for accidental switches and misdirection of care, but parents compensate by adopting new mechanisms for recognizing their offspring. Around the time that they begin to venture out from the nest, young swallows also develop individually distinct calls, which parents increasingly rely on for recognition. Once individual calls are established as the criteria for recognition, it is no longer enough to be in the right place at the right time; if they expect to be fed, young swallows must also be the right individuals.

Although context-dependent mechanisms can be effective at helping organisms direct altruism, they are also particularly susceptible to invasion by parasites. The cuckoo bird provides a classic example. Cuckoos lay their eggs in the nests of other bird species and then abandon them to the care of their unwitting foster parents. The cuckoo chick hatches from its egg and, in most cases, quickly dispatches its adopted brothers and sisters; when the parents return, they find the young cuckoo alone, assume that it is their last remaining offspring, and raise it to maturity. Such parasitic behavior clearly exploits parental assumptions about kinship identity and therefore provides a strong selective force against the maintenance of context-dependent recognition.

Because inter- and intraspecific parasitism exert such strong pressures on context-dependent systems, we might also expect organisms to supplement those systems with increasingly sophisticated recognition templates. Indeed, it appears that some bird species frequently parasitized by cuckoos have evolved mechanisms to discriminate between species-specific egg coloration patterns. Of course, this level of detection in turn exerts pressures on the eggs of the parasites themselves, leading to an evolutionary arms race that favors ever more subtle deception and detection. At least one species of brood parasite, the brown-headed cowbird, *Molothrus ater*, has evolved a dramatic strategy for dealing with this increased scrutiny. Adult cowbirds revisit parasitized nests on a regular basis, checking up on the eggs they left behind. If a host bird has been keen enough to discover a cowbird egg and eject it from its nest, the adult cowbird retaliates by destroying that nest and any eggs left inside. At this point, the behavior does not directly benefit the cowbird's

offspring, which have already been killed. It does, however, impose sharp costs on any host species capable of evolving more precise kin recognition systems. This not only slows the evolution of antiparasitic behaviors, but may also lead to behavioral changes within a single season: studies have shown that when some host birds rebuild their nests following retaliations, they become less discriminatory the second time around. In this way, the retaliation behavior is not just spiteful toward the host, but also directly beneficial to the individual cowbird's reproduction.

As these examples make clear, context-dependent recognition is far too precarious a strategy for some organisms, and this is especially true for those that live in large social groups without clear spatial boundaries. Most of these species have instead evolved template-based mechanisms for discriminating between kin and nonkin. One such mechanism, phenotype matching, is the subject of our next section.

The Persistence of Memory: Inferring Genetic Relationships Through Phenotype Matching

Many organisms recognize their kin through phenotype matching: a system in which individuals learn to associate specific sets of traits with varying degrees of kinship. The templates used in phenotype matching are established early in life, with reference either to family members encountered in the natal nest (where r is likely to be high) or to the individual's own phenotype (in which case $r=1$). The latter is a special case known as self-referent phenotype matching or, as Dawkins memorably put it, the armpit effect. We will return to it in more detail shortly.

Nestmate-based phenotype matching has some obvious advantages over the context-dependent mechanisms discussed earlier. For one thing, parasites hoping to escape detection must do more than simply show up in the right place at the right time; they must also evolve to mimic the genetically influenced traits required to pass for kin. Perhaps more importantly, phenotype matching allows individuals to assess the kin status of previously unencountered individuals. In social insects with large colonies and highly mobile workers, for example, this ability to assess never-before-encountered individuals is essential to determining who should be treated with care and who should be attacked. Obviously, neither context-dependent, spatially based recognition nor individual recognition would be sufficient in such large and complex populations.

While initial template formation may occur under similar circumstances in both context-dependent and phenotype matching recognition systems (e.g., 'this

individual is in my nest and therefore must be kin'), the durability of the latter allows recognition to take place long after the natal nest is abandoned. In fact, once a template is established, it is likely to remain intact for some time, often guiding that organism's recognition decisions throughout its life. Errors may still occur if templates are formed in the presence of unrelated individuals, but the window of opportunity for such mistakes is considerably smaller and limited to early development.

Organisms can presumably escape even this difficulty if they use the self-referent phenotype matching system mentioned earlier. If an individual can identify particular aspects of its own phenotype, use those aspects as referents for a kin recognition template, and then discriminate between other individuals on the basis of their similarities to that template, then that organism will possess a highly accurate means for identifying kin. Dawkins suggested the name 'armpit effect' because humans appear to generate individual-specific armpit odors which could, presumably, serve as referents for template formation. Although there is no direct evidence that this occurs in humans – dogs appear to be much better at smelling our individual differences than we are – the armpit effect has been successfully demonstrated in a handful of species. In one of the first studies of its kind, researchers tested whether or not pig-tailed macaques (*Macaca nemestrina*) could discriminate between previously unencountered paternal half-brothers and unrelated individuals. Since these paternal half-siblings neither grew up together nor had the same mothers, they could not have identified each other on the basis of phenotypes learned during early development; nevertheless, individual macaques showed a significant preference for half-siblings over unrelated individuals. This strongly implies that these monkeys were able to recognize paternally derived aspects of their own phenotype in their half-brothers and sisters, and the most likely mechanism for that recognition is self-referent phenotype matching.

The armpit effect has also been found in the golden hamster, *Mesocricetus auratus*, and in the honeybee, *Apis mellifera*. The latter case is especially interesting, as honeybee workers have strong incentives to match other individuals to their own phenotypes. Workers are nonreproductive and, through a peculiarity of Hymenopteran sex determination (haplodiploidy, in which females have two parents and males are fatherless), they are more related to their full-sisters ($r=0.75$) than they are to either their potential offspring or their mothers (both $r=0.5$). As such, honeybee society is particularly amenable to altruism between full-sister nestmates. This situation is complicated, however, by the polyandrous nature of the honeybee queen and by the multiple lines of paternity that exist in each hive. Again due to haplodiploid sex determination, workers are considerably less related to their half-sisters ($r=0.25$) than they are to either their full sisters or their mothers. Thus, altruistic behaviors

directed toward these half-sisters would not be favored and honeybees capable of discriminating between full- and half-sisters during nestmate care and queen rearing should be at an inclusive fitness advantage. Since these bees grow up surrounded by both full- and half-sisters, nestmate-based phenotype matching would not give individual workers the tools to make fine discriminations between patriline; it is not surprising, then, to find self-referent phenotype matching at work in these insects.

In the absence of carefully controlled studies, it is difficult to determine whether a particular phenotype matching system is based on nestmate- or self-referent formation. It is even more difficult, however, to discriminate between instances of the armpit effect and of so-called recognition alleles, genes that simultaneously code for both a phenotypic difference and an ability to recognize that difference in others. We will return to recognition alleles and to the closely related 'green-beard gene' concept shortly; first, we look more closely at the relationship between genes and phenotype matching in two well-studied systems: insects and mammals.

Smells of Home: Nest and Nestmate Odors in Insect Phenotype Matching

The first evidence for genetically influenced kin recognition in insects came in the late 1970s, when Greenberg demonstrated discrimination on the part of a primitively eusocial sweat bee, *Lasioglossum zephyrum*. In this study, bees standing guard at a nest entrance were introduced to previously unencountered individuals that varied by degree of kinship with the guards. Greenberg found that an individual's likelihood of making it past the guard was directly proportional to the coefficient of relatedness r between the two, regardless of a lack of past encounters; this implied that the bees were somehow able to determine relatedness in the absence of individual recognition or previous association. Drawing on previous studies, Greenberg concluded that the kinship signal was chemical in nature and that the bees were able to pick up on genetically determined odor differences between relatives and nonrelatives, presumably through phenotype matching.

In the years since, much of the research into insect kin recognition has focused on the role of olfaction and chemical cues, particularly those associated with cuticular hydrocarbons, or CHCs. The epicuticles of most insects are covered by these antidesiccation molecules, which vary between individuals and species in terms of both structure and relative abundance. This high variability makes CHCs obvious candidates for the expression component of kin recognition.

An impressive body of evidence does, in fact, point to a central role for CHCs in insect communication and

recognition, and most researchers now view them as the primary cues necessary for detecting kin. But this raises an interesting question: Are these chemical profiles intrinsic (i.e., specified by an individual's genes), environmentally acquired from nests or relatives, or some complex combination of the two? While studies have shown that some components of CHC profiles are heritable in male crickets, honeybees, and fruit flies, much remains unknown about other insect groups. Most likely, many insects develop their profiles through a complex interplay of both genetic and environmental factors.

In several groups of ants, for instance, each individual contributes its personal, intrinsic scent to a colony-wide, or 'gestalt,' odor that serves as a referent for template formation. Meanwhile, each ant acquires this complex gestalt odor on its cuticle, thus labeling itself as a member of its home colony. In some other species, colony-specific odors come exclusively from the queen, while in still others, the gestalt odor is based on a mixture of exclusively worker-derived odors. Some species use exogenous, environmental odors from foods or nest materials to supplement the chemical cues produced by the ants themselves, while others may rely exclusively on those intrinsic cues. The important point is that the relative contributions of genetics and of the social and physical environments vary widely between species and that, in most of those species, the details remain a mystery.

The use of chemical recognition cues has, however, been well studied in paper wasps of the genus *Polistes* and serves as an excellent example of the complex role of CHCs in recognition. Paper wasps are primitively eusocial insects that live in colonies dominated by a single reproductive female assisted by nonreproductive workers. According to Hamilton's rule, such altruistic division of labor is advantageous for worker genes only when helping behaviors are preferentially directed toward close relatives. It is in a worker's interest, then, to possess an accurate and effective kin recognition system.

Paper wasps appear to discriminate between kin and nonkin with the help of CHC profiles borne on the epicuticular surfaces of each individual. There is evidence to suggest that these profiles are influenced by both genetic and environmental factors and that the natal nest is the primary source of these odors. Wasps are much more readily accepted by individuals from the nest on which they eclosed (emerged from pupation) than by individuals from other nests; this holds true even when wasps are reared in nests where the average relatedness is low and nestmates are less-related to each other than to nonnestmates. The mechanism underlying this ability appears to be the acquisition of chemical cues embedded in the nest material itself. Indeed, studies have shown that paper wasps can discriminate between out-of-context fragments of their own and foreign nests, even in the absence of adults and brood. Recognition cues appear, then, to be homogenized

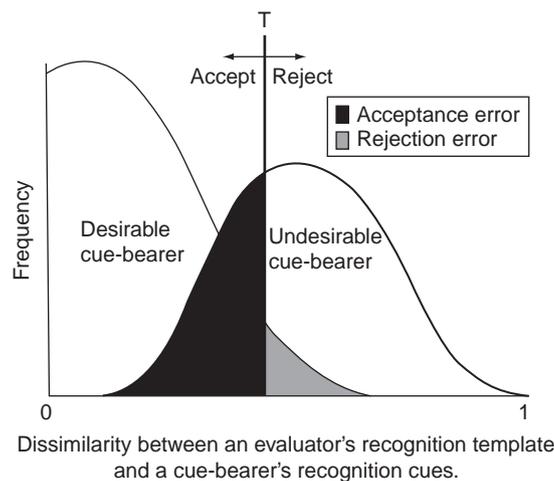
across nestmates and to employ nest of origin as an imperfect proxy for genetic relatedness.

Nest of origin also plays a critical role in the formation of *Polistes* recognition templates. Wasps tend to accept individuals raised on the same nest comb as themselves, while rejecting those raised on foreign nests. Again, this rule applies regardless of actual genetic relationship; in studies in which preeclusing wasps were switched to the nests of nonrelated females, wasps discriminated against their genetic relatives in favor of individuals from the same natal nest. The wasps apparently develop their recognition templates through a phenotype matching system using early, postemergence environmental cues as referents. It is worth pointing out, however, that individual wasps still appear to identify and use genetic cues when nest-origin cues are absent, hinting at a possible role for self-referent phenotype matching.

Polistes wasps respond to complex signals when evaluating kinship, but interestingly, do not appear to make subtle distinctions between kinship categories. During a specific recognition event – say an encounter between two previously unacquainted wasps – the evaluator compares the cue-bearer's odor profile to the colony-specific template. If the cue-bearer sufficiently matches the template, then she is accepted as a nestmate; if not, the evaluator responds to the perceived intruder with aggression. These recognition interactions appear to follow an 'all or none' rule, with cue-bearers neatly divided into kin and nonkin categories. Getz's 'kingram' concept provides a helpful framework for thinking about such dichotomous decision making in the face of highly variable cue information (see [Box 1](#)).

While CHC-based kin recognition requires a great deal of specificity on the part of cue-bearers, it is still susceptible to sophisticated mimicry by parasites. A recently discovered example involves chemical deception in a cuckoo wasp, *Hedychrum rutilans*, a highly specialized brood parasite of the European beewolf, *Philanthus triangulum*. The beewolf is itself a predator of honeybees; it attacks and paralyzes worker bees in the field, carries them to its underground nest, and lays eggs on their cuticles. These egg-carrying honeybees are temporarily stored just within the nest entrance, then deposited deeper in the underground burrow and sealed up inside.

Females of *H. rutilans* have evolved to take advantage of this food provisioning through a sophisticated suite of behaviors. Before the honeybee is taken inside the nest, the cuckoo wasp quickly deposits her own eggs on the bee's surface. When the beewolf returns to its nest, it carries the bee, its own egg, and the egg of its parasite into the brood chamber. The cuckoo wasp larvae emerge first and immediately set to devouring both the beewolf offspring and the paralyzed honeybee, thus increasing their own fitness at a considerable cost to that of their hosts. Normally, we might expect *P. triangulum* females to evolve mechanisms to detect the presence of

Box 1 Kingrams and the optimal acceptance threshold

Kingrams help us to visualize the recognition process and to better understand the role of thresholds in decision making. Organisms are often forced to make binary decisions in the face of complex and highly variable information. For instance, when deciding whether or not to accept a newly-encountered individual as kin, an organism must confront a great deal of intra-group variation. Not all relatives will share precisely the same set of identifying labels and some will no doubt match the evaluator's template more closely than others. Likewise, there will probably be some overlap between relatives and nonrelatives in terms of template dissimilarity. How do organisms make good decisions in the face of this uncertainty?

Many appear to do so through an optimal acceptance threshold, a point (T on the diagram above) at which increasing cue dissimilarity triggers rejection behavior. All individuals falling to the right of T are rejected as nonrelatives, while all those on the left of T are accepted. We expect organisms to position T such that fitness is maximized with respect to the relative costs of acceptance and rejection errors. For instance, when rejecting true kin is more costly than accepting imposters, the optimum acceptance threshold should move to the right.

H. rutilans-specific CHC profiles and tag 'contaminated' honeybees for removal. But this is apparently made considerably more difficult by chemical mimicry on the part of the cuckoo wasp: analysis of *H. rutilans* and *P. triangulum* CHC profiles found that the cuckoo wasps share more of their chemical signatures in common with beeswolves than they do with other *Hedychrum* species!

The use of CHCs as species- or individual-recognition cues, while common, is not universal in the insect world. For example, CHCs, while present in diurnal fireflies, appear to be absent in their nocturnal cousins. These night-flying species not only have a lower risk of desiccation (presumably making dehydration-resistant CHCs unnecessary), but also rely much more heavily on visual cues for mate evaluation and species recognition. Indeed, the flash signals in *Photinus* fireflies are surprisingly complicated and information-rich, and apparently compensate well for a lack of chemical cues.

To Smell a Rat: Phenotype Matching and the Genes of the MHC

As we have seen, phenotype matching is not limited to insect systems; many other organisms use similar mechanisms to establish and maintain recognition templates. In this section, we explore the likely role of phenotype matching in mammalian kin recognition, discuss its implications for inbreeding avoidance and nepotism, and focus on a likely source of referent cues, the family of genes known as the major histocompatibility complex or MHC.

Previous sections have emphasized the importance of kin recognition to the evolution of altruism and nepotism and, indeed, much of the early work on recognition focused on these behaviors. There is, however, at least one instance in which animals might benefit from avoiding close kin and from associating instead with unrelated individuals: that instance, of course, is mate choice.

Inbreeding between closely related individuals often exacts a heavy toll on fitness, as rare and deleterious recessive alleles are expressed to disastrous effect in the offspring. As such, organisms that recognize kin do well to avoid mating with close relations. Several decades of research have now shown that inbreeding avoidance is indeed an important consequence of kin recognition and a powerful force in the maintenance of cue diversity.

Much of the research on inbreeding avoidance in mammals has focused on the highly polymorphic genes of the MHC. This family of genes has long been the subject of intensive study, though more for its central role in the vertebrate immune system than for its use in kin recognition. The genes of the complex code for cell surface glycoproteins that help immune T cells differentiate between self and nonself; if a cell has been invaded by a pathogen, small fragments of the invader's protein are presented to T cells via a pocket on the MHC protein's surface, marking that cell for destruction. Medical researchers have an obvious interest in exploring the functions of these genes and so routinely keep inbred strains of laboratory mice differing only at the MHC. It was through a fortuitous observation of these mice's mating behavior that researchers first thought to investigate the role of such immunity genes in kin recognition.

Yamazaki and colleagues first conducted studies on MHC and mate choice in the late 1970s and found that mice preferentially mate with individuals that differ from them at these loci. Subsequent studies showed that mice could be trained to discriminate between urine samples from MHC-different individuals, indicating olfactory perception of either the gene products themselves or of secondary compounds linked to those gene products. These researchers also discovered a fascinating role for the MHC in the phenomenon known as pregnancy block, the spontaneous abortion of pregnancy that occurs when female mice are exposed to the scent of an unfamiliar male. Consistent with kin selection theory, the risk of pregnancy block is considerably increased when the second male varies from the first at MHC loci.

The importance of these genes for mouse kin recognition goes beyond inbreeding avoidance to a role in cooperation and altruism. Female mice often nest together, sharing nursing duties and other parental responsibilities. One study found that females preferred to nest with MHC-similar individuals, thus increasing the likelihood that helping behaviors will benefit close relatives and increase inclusive fitness. This sets up an interesting contest between opposing selection pressures, with sexual selection promoting MHC diversity (because individuals tend to prefer genetically dissimilar mates) and kin selection favoring greater homogeneity (through benefits accruing to MHC-similar individuals): a situation known as Crozier's Paradox. The extreme polymorphism found

at the MHC – Brown and Eklund suggest that in mice over three billion phenotypes are possible at just three principal loci – seems to imply that the effects of mate choice and sexual selection outweigh the selective forces applied by nepotism.

One area of research that has received particular attention and generated considerable controversy focuses on the role that MHC (also known as HLA) genes might play in human mate choice. Wedekind and colleagues' well-known 1995 study of odor preference in humans required females to smell a series of T-shirts worn by various males and to rank these shirts' scents according to pleasantness. The researchers found that the women were significantly more likely to deem an odor 'pleasant' if the shirt was worn by a man who differed at HLA loci. They were also more likely to report that such shirts reminded them of a current or former romantic partners, suggesting that pleasantness of odor and mate choice are related. Interestingly, preferences for HLA dissimilarity were reversed in women taking oral contraceptives at the time of the study.

Recent research seems to support the conclusion that HLA genotypes influence mate choice in humans. A large-scale study of Hutterite marriage patterns found that individuals were significantly more likely to marry partners with different HLA haplotypes than would be expected by chance. Similarly, a study testing odor preferences among Brazilian college students found that females preferred the smell of sweat from HLA-dissimilar males significantly more often than that from HLA-similar males; this study did not, however, find a similar effect when males ranked female sweat odors or when females ranked odors originating in male urine.

Much of the available evidence points to early childhood-based phenotype matching as the most likely source of these mating preferences. Yamazaki reversed odor preferences by rearing mice with MHC-different individuals: a situation unlikely to occur in nests in the wild. Several other studies and decades of anecdotal evidence indicate that humans learn relatives' phenotypes during childhood and later avoid those phenotypes when choosing mates.

Although a growing body of evidence supports a link between MHC genes and kin recognition, some researchers remain skeptical. They point out that the majority of mouse studies use highly inbred laboratory strains living under artificial conditions and thus may not shed much light on the behaviors of genetically diverse, wild populations. A recent study investigated the effects of MHC and MUP (major urinary protein) genotypes on inbreeding avoidance in mice living under natural conditions. Mice sharing both alleles at the MUP locus were significantly less likely to mate with each other than they were with mice who shared one or neither of their MUP alleles; the sharing of MHC alleles, however, had no significant effect on mating.

Another study of an unmanaged sheep (*Ovis aries*) population found no evidence of dissortative mating on the basis of MHC haplotype, suggesting that such genetic kin recognition may not be ubiquitous among mammals.

Not Easy Being Green: Super-Genes in Theory and in Nature

Workers of the invasive red fire ant, *Solenopsis invicta*, are not known for their civility. Fierce colony defenders, these tiny insects are quick to bite, grapple with, and sting, often to the point of death, any organism unlucky enough to wander into their nest mounds. Even so, it comes as a shock to watch a group of these workers descend on one of their own queens, overwhelm her with well-placed stings and bites, and literally rip her apart. What could possibly lead worker ants – organisms that depend on their queens as the sole sources of colony reproduction and inclusive fitness – to commit regicide?

The answer, it turns out, is a green beard. In his 1964 paper on the genetics of social behavior, William Hamilton described a hypothetical ‘super-gene’ (i.e., a tightly linked complex of several genes) that would simultaneously code for (1) a conspicuous phenotypic cue, (2) an ability to detect this cue in others, and (3) a propensity to direct care exclusively toward other cue-bearing individuals. Dawkins memorably dubbed such super-genes ‘green-beards,’ after an imaginary example in which beard color served as the recognition cue. It is just such a green-beard gene, and its recognition by *S. invicta* workers, that leads these ants to dispatch their queens with such surprising violence.

Colonies of *S. invicta* come in two forms, those with single queens (monogynous colonies) and those with multiple queens (polygynous colonies), and this difference in social structure corresponds to a genetic difference at a specific locus known as *Gp-9*. All queens and workers in monogynous colonies are dominant homozygotes (*BB*) at *Gp-9*, while all queens and most of the workers in the polygynous colonies are heterozygotes (*Bb*); homozygous recessive females are not found in the colonies because the *bb* genotype inevitably leads to death early in development.

The monogyne and polygyne populations are distinct and are connected genetically only through males who move between the two groups. In the polygyne colonies, matings between *Bb* females and *B* males (males are haploid) inevitably result in some new *BB* queens in every generation; without intervention, these *BB* queens would grow up to become reproductives and, year by year, their genotype would increase its representation in the colony. In reality, however, these new *BB* queens do not stand a chance: workers, with their penchant for political assassination, quickly intervene to maintain genetic disequilibrium. *Bb* workers can, through pheromonal cues, detect a

new queen’s genotype and modify their behavior accordingly. If she is heterozygous like them, she receives the typical protection due to a colony’s queen, but if she carries two copies of the *B* allele, she is marked for execution. The *Bb* workers surround the *BB* queen and swiftly eliminate her from the gene pool.

This is not exactly what biologists expected from a green-beard in nature. Recall that Hamilton’s original conditions specified altruism, not lethal violence, as the behavior associated with this degree of direct genetic recognition. But even though heterozygous *S. invicta* workers respond to the absence rather than the presence of a shared genotype, the effect remains the same. To return to Dawkins’s metaphor, imagine a set of closely linked alleles specifying both the possession of a green beard and an unusually high degree of aggression directed toward any individuals with purple beards. When green beards encounter other green beards, they treat each other with the normal degree of cooperation expected in the species. When they encounter purple beards, however, the latter suffers a significant reduction in fitness. This situation is analogous to that of the *S. invicta* workers and is functionally equivalent to the classic green-beard scenario described earlier.

The most surprising thing about the green-beard gene *Gp-9* is not that it results in aggressive behavior, but rather that it exists at all. Both Hamilton and Dawkins thought the likelihood of finding a green-beard in nature very small, because either (1) such super-alleles would be so successful that they would quickly fix themselves in a population and become invisible to analysis, or (2) once everyone in a population carried the conspicuous marker of genetic altruism, the system would become highly vulnerable to cheating. Individuals born with the marker (the green beard, say), but without the associated altruism, would benefit from the help of everyone else in the population without making sacrifices of their own. With such a fitness advantage, these individuals would quickly come to dominate the gene pool, and the benefits associated with the green beard would turn into handicaps. *Gp-9* avoids this fate at least in part because the *b* allele responsible for aggressive behavior can never become fully fixed in the population; its lethality in homozygous recessives prevents it from becoming the predominant allele and so the polygynous cycle of *BB* birth and elimination continues generation after generation.

So, contrary to predictions, green-beard genes do exist in nature. But are they common? As of now, the consensus answer is a qualified ‘no’ – very few examples have been discovered, the theoretical obstacles to their evolution still apply, and it is not immediately obvious how best to differentiate them from instances of the armpit effect mentioned earlier. But in the wake of research into the role of *Gp-9* in fire ants and of a possible green-beard effect at placental interfaces, researchers are more willing

than ever to consider a possible role for these super-genes in recognition systems.

One fairly unequivocal example comes from a species of unicellular slime mold, *Dictyostelium discoideum*. These social amoebas spend most of their time living as single-celled predators of bacteria, but when food gets scarce, they coalesce to form a multicellular colony known as a 'motile slug.' This cellular aggregation moves as a single unit until it comes to a suitable location on which to form a fruiting body, a distinctively shaped structure with reproductive spores sitting atop a long, thin stalk. Only those cells that find themselves at the top of this structure get the opportunity to escape into a better environment; the cells that make up the stalk inevitably die, sacrificing themselves for colony mates further up in the spores.

Since *D. discoideum* fruiting bodies are almost always composed of more than one clonal line and therefore contain individuals that vary widely in relatedness, the stage is set for kin selection. It appears that *D. discoideum* individuals bearing wild-type copies of a gene known as *csA* are able to respond to the presence of this allele in others and to preferentially direct cooperative behavior toward those individuals. *csA* codes for a self-binding (homophilic) protein that sits on the surface of *D. discoideum* cells, and this self-binding property causes wild-type individuals to adhere more readily to each other than they do to mutants bearing a different *csA* allele. The increased binding between these cells allows them to preferentially pull each other upward toward the top of the fruiting body, increasing their chances of survival and leaving mutant cells to die in the stalk.

The wild-type *csA* allele thus fulfills all the requirements for a green-beard gene. First, each cell bears a conspicuous cue in the form of a cell-surface adhesion protein; second, the homophilic nature of these proteins allows cells to recognize the genotype of another individual; finally, because these wild-type *csA* cells preferentially bind to and pull at genotypically similar individuals, the allele is able to direct care toward other copies of itself in the population. Indeed, the *csA* gene product may be only the first of many cell-surface adhesion proteins found to play a role in kin recognition. Because of their highly developed ability to discriminate between self and nonself, cell-surface adhesion proteins hold out particular promise as green-beard mechanisms.

Researchers have even speculated that green-beard genes and their surface adhesion products could influence sperm behavior in New World marsupials. In these animals, sperm cells swim in pairs, attaching to each other by their head segments and beating their flagella in concert. Together, they consistently out-swim any un-partnered rivals, but the alliance is an uneasy one because only one member of the pair typically goes on to fertilize an egg. If a gene could cause a sperm cell to partner exclusively with other cells carrying that same gene, then it could

ensure that at least one copy of itself would enter the egg. From this gene's point of view, the eventual competition between the two sperm cells would become no competition at all. While no evidence has yet been found to support the suggestion that green beards play a role in sperm partner choice, recent discoveries in *Solenopsis* and *Dictyostelium* show that such a mechanism is far from improbable.

It is important to point out that not all recognition alleles are green-beards *sensu stricto*. The former definition only requires that a gene create a conspicuous cue and the ability to detect that cue in others; the latter, meanwhile, requires both of those conditions plus behaviors favoring the cue-bearers. The key difference is that a green-beard gene carries all three phenotypic effects at a single locus or tightly linked set of loci, while some recognition alleles may rely on behaviors coded for at other locations in the genome. In the case of *Botryllus schlosseri*, a marine tunicate, individual colonies are able to recognize kin on the basis of genetic similarity at a single locus, the *FuHC* gene. Sessile colonies often grow into each other across a shared substrate. If individuals of the colonies share one or both of their alleles (out of literally hundreds available), then the colonies easily fuse together; when they share neither, the colonies reject each other with an aggressive inflammatory response. This example, along with similar ones from hydrozoans and plants, does not imply the existence of green-beards, as the accompanying behavior is not a result of the *FuHC* gene itself. Instead, this gene serves as a source of recognition cues and as a mechanism for comparing those cues to self, in other words, as a recognition allele.

Finally, it is worth asking whether or not green-beard alleles are really kin recognition mechanisms at all. To return once more to Dawkins's beards, a *DD* green-bearded individual may well grow up in a household with a *dd* red-bearded sibling (if parental cross is *Dd* × *Dd*). Given the choice between helping a green-bearded second cousin or a red-bearded brother, the individual in question would prefer to assist the former every time. In this way, green-beard super-genes help themselves not only at the expense of the individual's fitness, but also at the expense of the other genes with which they share a body.

Conclusions and Future Directions

Forty years ago, Hamilton's insights lay the groundwork for an explosion of kin recognition research; today, the rapid development of modern genetic techniques is building a new foundation, this time for understanding how genes interact with the environment to build kin recognition mechanisms. The examples given in this article illustrate both the interdisciplinary nature of the work and the breadth of strategies and mechanisms that animals use to recognize kin. Future contributions from the fields of

animal behavior, neurobiology, and cellular biology will only expand this understanding and may well challenge many of our current assumptions.

See also: Kin Selection and Relatedness; Recognition Systems in the Social Insects; Social Recognition.

Further Reading

- Blaustein AR (1983) Kin recognition mechanisms: Phenotypic matching or recognition alleles? *The American Naturalist* 121: 749–754.
- Brown JL and Eklund A (1994) Kin recognition and the major histocompatibility complex: An integrative review. *The American Naturalist* 143: 435–461.
- Crozier RH and Dix MW (1979) Analysis of two genetic models for the innate components of colony odor in social hymenoptera. *Behavioral Ecology and Sociobiology* 4: 217–224.
- Dawkins R (1982) *The Extended Phenotype*. Oxford: Oxford University Press.
- Fletcher DJC and Michener CD (eds.) (1987) *Kin Recognition in Animals*. New York, NY: John Wiley & Sons.
- Greenberg L (1979) Genetic component of bee odor in kin recognition. *Science* 206: 1095–1097.
- Hamilton WD (1964) The genetic evolution of social behaviour I, II. *Journal of Theoretical Biology* 7: 1–52.
- Hepper PG (ed.) (1991) *Kin Recognition*. Cambridge: Cambridge University Press.
- Keller L and Ross KG (1998) Selfish genes: A green beard in the red fire ant. *Nature* 394: 573–575.
- Mateo JM and Johnston RE (2000) Kin recognition and the 'armpit effect': Evidence of self-referent phenotype matching. *Proceedings of the Royal Society of London B* 267: 695–700.
- Rousset F and Roze D (2007) Constraints on the origin and maintenance of genetic kin recognition. *Evolution* 61: 2320–2330.
- Sherman PW, Reeve HK, and Pfennig DW (1997) Recognition systems. In: Krebs J and Davies N (eds.) *Behavioural Ecology: An Evolutionary Approach*. Oxford: Wiley-Blackwell.
- Starks PT (ed.) (2004) Recognition systems (special issue). *Annales Zoologici Fennici* 41: 689–892.
- Strohm E, Kroiss J, Herzner G, et al. (2008) A cuckoo in wolves' clothing? Chemical mimicry in a specialized cuckoo wasp of the European beewolf (Hymenoptera, Chrysididae and Crabronidae). *Frontiers in Zoology* 5: 2.
- Wedekind C, Seeback T, Bettens F, and Paepke AJ (1995) MHC-dependent mate preferences in humans. *Proceedings of the Royal Society of London B* 260: 245–249.