

Kin Recognition in Protists and Other Microbes

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*Genetics, Evolution,
Behavior and Health*

By

Guillermo Paz-y-Miño-C
and Avelina Espinosa

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PREFACE

In the *Origin of Species* (1859), Charles Darwin speculated about the puzzle of the sterile social insects, in which female workers at a nest dedicate their lives to the persistence of the colony (structured around a large progeny), via assisting a fertile queen to reproduce with the available males. Darwin implied that, in such cases of apparent sacrifice —by the workers— for the good of all (i.e. the "community," *sensu stricto*), "selection may be applied to the family."

In Chapter VII of *The Origin*, Darwin rationalized about two topics concerning sterile social ants and bees: "difficulties on the theory of the natural selection of instincts" and "neuter or sterile insects." Darwinianly he wrote:

"...[I] will confine myself to one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory [of natural selection]. I allude to the neuters or sterile females in insect-communities [=colonies]: for these neuters often differ widely in instinct [=behavior] and in structure [=anatomy] from both the males and fertile females, and yet, from being sterile, they cannot propagate their kind... How the workers have been rendered sterile is a difficulty; but not much greater than that of any other striking modification of structure; for it can be shown that some insects and other articulate animals [=arthropods] in a state of nature occasionally become sterile; and if such insects had been social, and it had been profitable to the community that a number should have been annually born capable of work, but incapable of procreation... [then the] difficulty... is lessened, or, as I believe, disappears, when it is remembered that *selection may be applied to the family*, as well as to the individual, and may thus gain the desired end... I have such faith [=confidence] in the powers of selection, that I do not doubt that... a slight modification of structure, or instinct, correlated with the sterile condition of certain members of the community, has been advantageous to the community: consequently the fertile males and females of the same community flourished, and transmitted to their fertile offspring a tendency to produce sterile members having the same modification. And I believe that this process has been repeated, until that prodigious amount of

difference between the fertile and sterile females of the same species has been produced, which we see in many social insects..."

In the late 1800s, Darwin could not offer a heredity-based explanation for "selection [being] applied to the family." Ronald A. Fisher (1930) and John B. S. Haldane (1932, 1955) wrestled with the genetics and mathematics of altruism and the anecdotic expression "I would lay down my life for two brothers or eight cousins" became legacy of their work. William D. Hamilton (1963, 1964) and John Maynard-Smith (1964) further reasoned that the ability to discriminate between close and distant genetic relatives could be directly linked to survival and reproductive success, and, ultimately, to kin selection (as per Maynard-Smith 1964, 1977). Close relatives would engage in altruistic cooperation to pass on the shared genes and minimize competition with kin. According to Hamilton (1963, 1964), the fitness benefits (b) of helping another individual correlate with the coefficient of genetic relatedness (r) and are closely dependent on the costs (c) of helping. If the benefits (b) surpass the costs (c), then altruistic cooperation could evolve ($b - c > 0$).

Hamilton's model suggested that prior to engaging in adaptive cooperation or altruism among close relatives, organisms would have to detect kin and do it, ultimately, via a gene or genes (i.e. a mechanism later called "recognition alleles"). In retrospect, his "super gene" model referred to a pleiotropic loci capable of: (1) influencing the expression of a trait for recognition, which would allow (2) carriers of the trait to recognize similar traits in others, and (3) induce the carriers to behave altruistically only toward other carriers of the gene. Richard Dawkins (1976) called this phenomenon the "green-beard effect" (i.e. green-beard genes) in which any carrier of the green-beard-cue for recognition would spot the cue in conspecifics (we examine the pros and cons of Hamilton's and Dawkins' premises in Chapter Two of this book).

Most of the theoretical framework and experimental settings to elucidate the mechanisms of kin recognition/discrimination come from studies with multicellular eukaryotes, particularly insects, amphibians, fish, reptiles, birds, mammals and, to a much lesser extent, plants (chronological reviews in Holmes and Sherman 1983; Fletcher and Michener 1987; Blaustein et al. 1988; Hepper 1991; Sherman et al. 1997; Tang-Martínez 2001; Starks 2004; Biedrzycki and Bais 2010; Penn and Frommen 2010; Dudley et al. 2013; Paz-y-Miño-C and Espinosa 2016). Theory and laboratory work have relied on the assumption that altruistic, nepotistic

behavior —adaptive cooperation within family units— has evolved in parallel with an organism's ability to tell apart close- from distant- or non-related conspecifics (Chapter One of this book).

The "field of kin recognition," which conceptual foundations can —thus— be traced back to the 1960s, has no consensus on definitions or proposed mechanisms (Chapters One, Two and Three; Espinosa and Paz-y-Miño-C 2014; Paz-y-Miño-C and Espinosa 2016), possibly due to the vast diversity and complexity of life histories across the organisms that have been most studied: animals and plants. Only recently, unicellular eukaryotes, or protists, and other microbes (prokaryotes: bacteria and archaea), have been included in these investigations. They are the focus of this book.

In *Kin Recognition in Protists and Other Microbes* we refer to "recognition" as an organism's ability to identify kin versus non-kin (and do it differentially as per relatedness, $r = 1.0$ to 0.0); in addition, we use the term "discrimination" as the capacity to distinguish one cell line (clone) from another, or "same" from "different." Because we discuss instances of taxa-, clone-, and kin-discrimination/recognition in single-celled organisms capable of both discriminating between same and different and discriminating/recognizing among clones of distinctive value of r , we use these terms together. But colleagues specializing on these topics might disagree with us and argue that re-cognition involves a "cognitive" process (re-calling) not present in all organisms, which is semantically true (note that this very argument has been debated in numerous reviews; Chapters One, Two and Three; Blaustein et al. 1988; Sherman et al. 1997; Tang-Martínez 2001; Starks 2004; Penn and Frommen 2010). We use discrimination/recognition together also for practical, didactic reasons: to refer to the discrimination processes (i.e. distinction between same and different taxa or clones; self/non-self; or kin/non-kin) and in association with the history of the field of kin recognition, an approach we reinforce along the book.

This work is the first in which taxa-, clone- and kin-discrimination/recognition in unicellular eukaryotes (protists) and other microbes is organized from a historical perspective (i.e. the advent of protists and microbial models in the field of kin recognition; Chapters One and Five). We discuss: the genetics of kin discrimination/recognition in unicellular organisms, including green-beard-gene polymorphisms in social amoebas, yeast and bacteria (Chapters Two and Five); the potential that microbes have to learn

phenotypic cues during socio-sexual encounters and use such decoded information adaptively in behavioral responses (Chapter Three); the exchange of chemical signals, often released into the environment, and used for taxa-, clone- or kin-discrimination/recognition in amebas, ciliates and soil bacteria (Chapters Three, Four and Five); the relevance of clonality and kinship for pathogenicity, particularly in *Entamoeba*, *Plasmodium* and *Trypanosoma*, and for biofilm formation in the bacteria *Escherichia*, *Pseudomonas*, *Staphylococcus* and *Vibrio* (Chapters Four, Five and Six); the correlations between kinship, social structure, spatial distribution and micro-biogeography at local, regional and continental scales, as well as at microscopic levels (Chapters Five and Seven); the relevance of protists' and other microbes' cell aggregations, cooperation, sociality and cheating (or avoidance of it) for our understanding of the origins and evolution of multicellularity (Chapters Five and Eight); and the directions that the field of kin-discrimination/recognition shall take in the future now that microbes are increasingly being studied —under such perspective— in the laboratory and field (Chapter Nine).

The book has a genetic, evolutionary and behavioral approach and it has been conceptualized for a broad audience, including researchers in academia (microbial genetics, evolution, protistology, animal behavior, integrative biology, microbial ecology and health), post doctoral fellows, graduate students and research undergraduates. We think that science writers and college educators shall find the volume informative (it includes 120+ figures and 200+ sub-figures). The length and chapter organization make the book suitable for a graduate seminar and/or laboratory discussions facilitated by a principal investigator; keep in mind that some chapters are extensive (e.g. Five and Seven). Because the chapters increase in complexity, we recommend the reader to explore them in order. However, we have also organized the material to be read independently by chapter (by the more experienced reader). For that reason, along the book we deliberately reinforce concepts and summarize content (e.g. meaning of acronyms, synopsis of figures, tables and data from previous sections) and frequently advise the reader to explore figures in a specific manner. We include dialog boxes to highlight the central message of figures and/or expedite the interpretation of the statistics.

Guillermo Paz-y-Miño-C
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CHAPTER ONE

KIN RECOGNITION: SYNOPSIS AND THE ADVENT OF PROTISTS MODELS

Most of the theoretical framework and experimental settings to elucidate the mechanisms of kin recognition/discrimination come from studies with multicellular eukaryotes (i.e. insects, amphibians, fish, reptiles, birds, mammals and, to a much lesser extent, plants; Holmes and Sherman 1983; Fletcher and Michener 1987; Blaustein et al. 1988; Hepper 1991; Tang-Martínez 2001; Biedrzycki and Bais 2010; Penn and Frommen 2010; Dudley et al. 2013). Theory and laboratory work have relied on the assumption that altruistic, nepotistic behavior —adaptive cooperation within family units (Box 1.1)— has evolved in parallel with an organism's ability to tell apart close- from distant- or non-related conspecifics.

Box 1.1. Essential kin-recognition terminology.

There is no consensus on definitions, concepts or proposed mechanisms in the field of kin recognition/discrimination. Our aim here is to facilitate the comprehension of the topics discussed in this chapter and book by clarifying the way in which we use some terms (in-depth reviews in Sherman et al. 1997; Tang-Martínez 2001; Starks 2004; Penn and Frommen 2010).

Altruistic behavior. An action in which an individual (the actor) reduces its/his/her fitness at the same time as the action increases the fitness of others (the recipients, usually kin).

Behavior. Anything and everything an organism does and the way it/he/she does it. In the context of this chapter and book, behavior includes any and every action, or reaction, in a kin-recognition/discrimination situation.

Discrimination. The ability of an organism to distinguish same from different, or to tell individuals (or their aggregations) apart; e.g. one clone (coefficient of genetic relatedness $r = 1.0$) from another clone ($r \approx 0.0$).

Fitness. The overall genetic legacy of an individual to future generations. An individual can accomplish this via its/his/her own reproduction (direct fitness) or via helping others (usually kin members) to reproduce (indirect fitness).

Kin recognition. The ability of an organism to identify kin versus non-kin. It includes the ability to respond differentially (via behavior) to distinctive degrees of genetic proximity; e.g. clone ($r = 1.0$), sibling ($r = 0.5$), or other related ($r = 0.25, 0.125$) or non-related conspecifics ($r \approx 0.0$). For "kin vs. kind" see Chapter Five; for "kind" discrimination/recognition see Box 5.2.

Kin selection. KS relies on individual selection and refers to situations in which close genetic relatives (kin) have a survival and reproductive advantage in respect to other individuals (and perhaps even other kin units) in a population. Such advantage comes from indirect fitness gains (e.g. altruistic cooperation, nepotistic behavior). Criticisms to KS highlight that altruism/nepotism are apparent and rely, ultimately, on individual selfishness to "help" kin to survive and reproduce when the individual has no other possibility to pass on its/his/her own genes to future generations (review in Birch and Okasha 2014). For "kind" selection see Box 5.2 in Chapter Five.

Nepotistic behavior. Actions in which an individual organism, or groups, favor the survival and reproduction of close genetic relatives. Nepotistic behaviors can also benefit non-kin, particularly among organisms in which social alliances have been consolidated over time (friendships); but the term nepotism is more frequently used in reference to kin.

Darwin (1859) speculated about the puzzle of the sterile social insects, in which female workers at a nest dedicate their lives to the persistence of the colony (structured around a large progeny), via assisting a fertile queen to reproduce with the available males. Darwin implied that, in such cases of apparent sacrifice —by the workers— for the good of all (i.e. the "community," *sensu stricto*), "selection may be applied to the family." But, in the late 1800s, he could not offer a heredity-based explanation for the latter (Paz-y-Miño-C and Espinosa 2016; see Box 1.2 for a summary of Darwin's conjectures about how natural selection, at the family level, would shape social structure, reproduction and sterility in social insects).

Box 1.2. Darwin and the puzzle of the sterile social insects.

In Chapter VII of the *Origin of Species* (1859), Charles Darwin rationalized about two topics concerning sterile social ants and bees (i.e. "difficulties on the theory of the natural selection of instincts" and "neuter or sterile insects"):

"...[I] will confine myself to one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory [of natural selection]. I allude to the neuters or sterile females in insect-communities [=colonies]: for these neuters often differ widely in instinct [=behavior] and in structure [=anatomy] from both the males and fertile females, and yet, from being sterile, they cannot propagate their kind.

[Here I will] take only a single case, that of working or sterile ants. How the workers have been rendered sterile is a difficulty; but not much greater than that of any other striking modification of structure [=an anatomical trait]; for it can be shown that some insects and other articulate animals [=arthropods] in a state of nature occasionally become sterile; and if such insects had been social, and it had been profitable to the community [=colony] that a number should have been annually born capable of work, but incapable of procreation, I can see no very great difficulty in this being effected by natural selection... The great difficulty lies in the working ants differing widely from both the males and the fertile females in structure [=anatomy], as in the shape of the thorax and in being destitute of wings and sometimes of eyes, and in instinct [=behavior]. As far as instinct alone is concerned, the prodigious difference in this respect between the workers and the perfect females [=the reproductive females], would have been far better exemplified by the hive-bee. If a working ant or other neuter insect had been an animal in the ordinary state, I should have unhesitatingly assumed that all its characters had been slowly acquired through natural selection; namely, by an individual having been born with some slight profitable modification of structure [=anatomy], this being inherited by its offspring, which again varied and were again selected, and so onwards. But with the working ant we have an insect differing greatly from its parents, yet absolutely sterile; so that it could never have transmitted successively acquired modifications of structure [=anatomy] or instinct [=behavior] to its progeny. It may well be asked how is it possible to reconcile this case with the theory of natural selection?

...[L]et it be remembered that we have innumerable instances, both in our domestic productions [=selective breeding of animals or plants] and in those in a state of nature [=animals or plants in the wild], of all sorts of differences

of structure [=anatomy] which have become correlated to certain ages, and to either sex. We have differences correlated not only to one sex, but to that short period alone when the reproductive system is active, as in the nuptial plumage of many birds, and in the hooked jaws of the male salmon. We have even slight differences in the horns of different breeds of cattle in relation to an artificially imperfect state of the male sex; for oxen of certain breeds have longer horns than in other breeds, in comparison with the horns of the bulls or cows of these same breeds. Hence I can see no real difficulty in any character having become correlated with the sterile condition of certain members of insect-communities: the difficulty lies in understanding how such correlated modifications of structure could have been slowly accumulated by natural selection.

This difficulty, though appearing insuperable, is lessened, or, as I believe, disappears, when it is remembered that *selection may be applied to the family*, as well as to the individual, and may thus gain the desired end... I have such faith [=confidence] in the powers of selection, that I do not doubt that a breed of cattle, always yielding oxen with extraordinarily long horns, could be slowly formed by carefully watching which individual bulls and cows, when matched [for reproduction], produced oxen with the longest horns; and yet no one ox could ever have propagated its kind. Thus I believe it has been with social insects: a slight modification of structure [=anatomy], or instinct [=behavior], correlated with the sterile condition of certain members of the community [=colony], has been advantageous to the community: consequently the fertile males and females of the same community flourished, and transmitted to their fertile offspring a tendency to produce sterile members having the same modification. And I believe that this process has been repeated, until that prodigious amount of difference between the fertile and sterile females of the same species has been produced, which we see in many social insects..."

Fisher (1930) and Haldane (1932, 1955) wrestled with the genetics and mathematics of altruism and the anecdotic expression "I would lay down my life for two brothers or eight cousins" became legacy of their work. Hamilton (1963, 1964) and Maynard-Smith (1964) further reasoned that the ability to discriminate between close and distant genetic relatives could be directly linked to survival and reproductive success, and, ultimately, to kin selection (as per Maynard-Smith 1964, 1977; but see Box 1.1). Close relatives would engage in altruistic cooperation to pass on the shared genes and minimize competition with kin. According to Hamilton (1963, 1964), the fitness benefits (b) of helping another individual correlate with the coefficient of genetic relatedness (r) and are closely dependent on the

costs (c) of helping. If the benefits (b) surpass the costs (c), then altruistic cooperation could evolve ($b - c > 0$; for expansion, see Box 1.3).

Box 1.3. Hamilton and the concepts of fitness.

In the 1960s, William D. Hamilton explained that individual organisms could maximize their genetic legacy via both their own reproduction (i.e. the number of fertile offspring an individual is capable of producing, or its/his/her direct fitness) and via assisting close genetic relatives (e.g. kin members at a nest or colony) to reproduce and pass on the shared genes to the descendants (indirect fitness). The term inclusive fitness (Hamilton 1963, 1964) refers to the total genetic contribution an individual makes to future generations via direct and indirect fitness (Gardner et al. 2016).

Thus, altruistic cooperation or nepotistic behavior (see terminology in Box 1.1) rely on indirect fitness gains in which close genetic relatives (kin) are the beneficiaries. Kinship offers a possible scenario (not the only one, see Chapters Five and Eight) for the evolution of long-lasting or permanent associations among individual organisms (multicellular or unicellular) in which the payback for cooperating surpasses the costs (i.e. the genetic legacy attained by helping kin members to reproduce exceeds the helper's costs of postponing its/his/her reproduction or not reproducing at all).

This suggests that to cooperate with kin and minimize investing on non-adaptive cooperation (errors) with distantly related or non-related conspecifics, organisms need mechanisms for discrimination and/or recognition of kin. And also strategies to identify and deter cheaters that might benefit from receiving help, or cooperation, without reciprocating (Chapter Eight; reviews in West and Gardner 2010; Gardner et al. 2016).

In Chapter Eight, we discuss how inclusive fitness relates to understanding cell aggregations among unicellular eukaryotes and their capacities to discriminate between same and different (i.e. taxa, clone, kin). Cell-cell recognition ability was likely crucial for the origin and evolution of long-lasting cell associations and multicellularity (Chapters Five and Eight).

The "field of kin recognition," which conceptual foundations can be traced back to the 1960s, has no consensus on definitions (Box 1.1.) or proposed mechanisms (Chapters Two and Three), possibly due to the vast diversity and complexity of life histories across organisms (reviews in Tang-Martínez 2001; Penn and Frommen 2010). In this book, we refer to "recognition" as an organism's ability to identify kin versus non-kin; in addition, we use the term "discrimination" as the capacity to distinguish

one clone from another. Because we discuss instances of taxa-, clone-, and kin-discrimination/recognition in single-celled organisms capable of both discriminating between same and different, and discriminating/recognizing among clones of distinctive value of r , we use these terms together (but see Tang-Martinez 2001; Penn and Frommen 2010).

The Advent of Protists Models

Recent studies with unicellular eukaryotes (protists) have uniquely enriched the field of kin recognition (Fig. 1.1), particularly after characterizing the genes involved in discrimination-mediated aggregation (mostly for flocculation or biofilm-like formation, starvation-triggered dormancy, or reproduction) or in clone-versus-clone competition to colonize hosts in parasitic taxa (Espinosa and Paz-y-Miño-C 2014a; Espinosa et al. 2016; Paz-y-Miño-C and Espinosa 2016). The last two decades of kin recognition studies (2000s onwards) with protists have been focused on the molecular biology and genetics of cell-cell discrimination and kin recognition abilities in *Saccharomyces*, *Dictyostelium*, *Polysphondylium*, *Tetrahymena*, *Entamoeba* and *Plasmodium* (Table 1.1; Espinosa and Paz-y-Miño-C 2014a; Paz-y-Miño-C and Espinosa 2016).

In later chapters of this book, we review these studies and remark on the type of scientific progress (i.e. basic and applied science) that can be achieved by using protists as model organisms in investigations of taxa, clone and kin discrimination. We highlight that protists are robust systems to test multiple hypotheses in overlapping research programs in kin recognition, which can also be extended to exploring the origins of multicellularity. Some of these hypotheses include: recognition alleles and "green-beard" genes (Hamilton 1964; Dawkins 1976); the communication problem in honest signaling for kin recognition (Johnstone 1997); cheating and concealed identity by signal-senders during kin recognition (anti-recognition strategies; Beecher 1991); origin of multicellularity under the inclusive fitness principle (Hamilton 1964), or as a byproduct of the interactions between cheaters and resisters in situations of low-genetic relatedness (Buss 1987; Levin et al. 2015). We discuss these topics from the perspective that an integrated, multidisciplinary approach (i.e. molecular, genetic, physiological, behavioral, ecological and evolutionary) is needed to make significant progress in the field. Realize, however, that the very field of social evolution is currently being reevaluated under integrative approaches (e.g. association theory; Gilbert 2017).

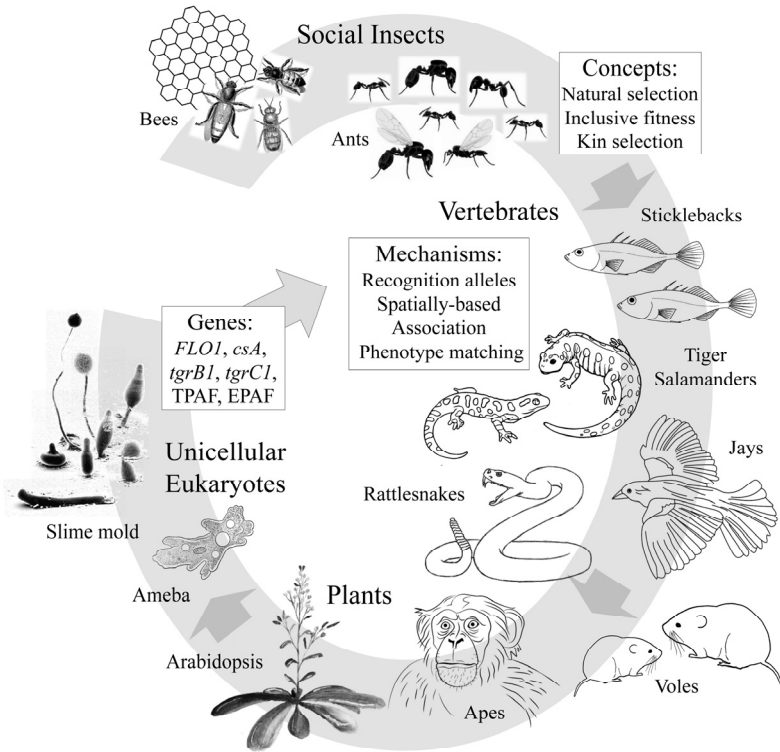


Fig. 1.1 The advent of protists models in the field of kin recognition/discrimination. The theoretical framework and experimental settings to elucidate the natural history and evolution of kin recognition/discrimination initially came from studies with multicellular eukaryotes, particularly social insects (bees, wasps), and later vertebrates and some plants. The concepts of natural selection (a mechanism itself), in the 1850s (Darwin's work; Box 1.2), inclusive fitness (Hamilton 1963, 1964; Box 1.3) and kin selection (Maynard-Smith 1964, 1977) became the foundation of kin recognition/discrimination at the mechanistic level (i.e. recognition alleles, spatially-based recognition, association and phenotype matching; for details see Chapters Two and Three). During the 2000s, studies with protists have focused on the molecular biology and genetics of cell-cell recognition/discrimination (gene acronyms and proliferation activating factors PAFs are detailed in Table 1.1). This approach has nurtured previous knowledge (indicated by central arrow) and ignited interest in expanding the field toward the study of the origins and evolution of cell-cell- associations, cooperation, multicellularity, and cheating. Figure design G. Paz-y-Miño-C (it includes collage of public domain www images, see Appendix A for sources).

Table 1.1 Evidence of taxa-, clone-, and kin-discrimination in protists (adapted from Espinosa and Paz-y-Miño-C 2014a; Paz-y-Miño-C and Espinosa 2016)

Organisms	Behavioral trait reported	Experimental observation	Genes involved
Dikarya <i>Saccharomyces cerevisiae</i>	Flocculation biofilm-like clusters	<i>FLO1</i> + cells cluster with carries of gene	<i>FLO1</i> gene ^a
Mycetozoa <i>Dictyostelium discoideum</i>	Fruiting-body formation	<i>csA</i> + cells form fruiting bodies with same	<i>csA</i> gene ^b
<i>D. discoideum</i>	Fruiting-body formation	Highly related (<i>r</i>) fruiting-body formation	Unknown ^c
<i>D. discoideum</i>	Mound formation, slug migration	Clonal aggregation/migration in cultures	<i>lagB1 lagC1</i> genes ^d
<i>D. discoideum</i>	Fruiting-body formation	Clonal fruiting bodies form in mixed cultures	<i>tgrB1 tgrC1</i> genes ^e
<i>D. purpureum</i>	Fruiting-body formation	Highly related (<i>r</i>) fruiting-body formation	Unknown ^f
<i>D. giganteum</i>	Fruiting-body formation	Clonal/non-clonal fruiting body formation	Unknown ^g
<i>Polysphondylium violaceum</i>	Fruiting-body formation	Clonal fruiting bodies form in mixed cultures	Unknown ^h
Ciliophora <i>Tetrahymena thermophila</i>	Aggregation in clusters	Motility toward and aggregation with clones	TPAF molecules ⁱ
Archamoebae <i>Entamoeba invadens</i>	Aggregation in clusters	Clonal aggregation in mixed cultures	Unknown ^j
<i>E. moshkovskii</i>	Aggregation in clusters	Clonal aggregation in mixed cultures	EPAF molecules ^k
Apicomplexa <i>Plasmodium chabaudi</i>	Among-clone competition	Selfing to outcompete unrelated	Unknown ^l
<i>P. falciparum</i>	Within-clone competition	Kinship patterns of infection in host	Unknown ^m

^aSmukalla et al. (2008); ^bQueller et al. (2003); ^cOstrowski et al. (2008); ^d*lag* and *tgr* are synonymous for the genes *lagB1/lagC1* and *tgrB1/tgrC1*; Benabentos et al. (2009). There are other genes involved in social cooperation in *D. discoideum*, however, indirectly linked to cell-cell discrimination/recognition, including: *fbxA* (targets proteins for ubiquitination), *dimA bZIP/bRLZ* (transcription factor); *chtC* (putative transmembrane protein, null mutants are facultative cheaters), *rcca*

(resists exploitation by *chtC* and does not cheat on wild type; Li and Purugganan 2011); ^eHirose et al. (2011); ^fMehdiabadi et al. (2006); ^gKaushik et al. (2006); ^hKalla et al. (2011); ⁱ*Tetrahymena* Proliferation Activating Factors TPAFs; Chaîne et al. (2010); ^jEspinosa and Paz-y-Miño-C (2012); ^k*Entamoeba* Proliferation Activating Factors EPAFs; Espinosa and Paz-y-Miño-C (2014a, b). Our lab has identified six putative cell-signals excreted by the amebas (RasGap/Ankyrin, coronin-WD40, actin, protein kinases, heat shock 70, and ubiquitin) and which known functions in *Entamoeba* spp. included cell proliferation, cell adhesion, cell movement, and stress-induced encystation; these putative EPAFs are likely linked to clone-clone discrimination/recognition (Espinosa et al. 2016); ^lReece et al. (2008); ^mNkhoma et al. (2012).

Note that important scientific literature exists on kin discrimination/recognition in prokaryotes and its implications for the origin and evolution of multi-cellular-prokaryotic assemblages. Although we discuss prokaryotes in Chapter Five, we do it succinctly, therefore, we advise the reader to search for information elsewhere (e.g. West et al. 2006; Kraemer and Velicer 2011; Rumbaugh et al. 2012; Celiker and Gore 2013; Mitri and Foster 2013; Pathak et al. 2013; Lyons and Kolter 2015).

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CHAPTER TWO

THE GENETICS OF KIN RECOGNITION: FROM MANY CELLS TO SINGLE CELLS

William D. Hamilton (1963, 1964) suggested that recognition of conspecifics—prior to engaging in adaptive cooperation or altruism among close relatives— would ultimately depend on genes (i.e. recognition alleles; see definitions in Box 2.1). In retrospect, his "super gene" model referred to a pleiotropic gene capable of: (1) influencing the expression of a trait for recognition, which would allow (2) carriers of the trait to recognize similar traits in others, and (3) induce the carriers to behave altruistically only toward other carriers of the gene (for theoretical context see Box 1.3 in Chapter One: *Hamilton and the concepts of fitness*). Dawkins (1976) called this phenomenon the "green-beard effect" (i.e. green-beard genes) in which any carrier of the green-beard-cue for recognition would spot the cue in conspecifics (regardless of their genetic proximity in the rest of the genome; Gardner and West 2010). The major problem with this model was that it would be evolutionarily unstable and prone to erode cue diversity (i.e. "Crozier's Paradox," Crozier 1986; but see Holman et al. 2013; Ho and Shaulsky 2015), since the popular cue (i.e. the green beard) would quickly outnumber other cues in the population and become ineffective for the high advantage it initially had: selective cooperation and altruism toward the carriers of the super gene(s). Alternatively, green-beard genes could be polymorphic, or multiple genes could potentially adopt comparable functions (for recognition), and the system become evolutionarily stable under the relative adaptive value of such genes in their genomes and populations (Gardner and West 2010).

Box 2.1. Essential terminology for this chapter.

Biofilm. Microbial aggregations of single- or multiple-prokaryotic taxa (i.e. communities) embedded in their own extracellular secretions or Extra Polymeric Substances (EPS). EPS are made of poly-chains of carbohydrates, proteins, lipids and nucleic acids. Associations of cells coated by EPS can

access and metabolize resources efficiently, cooperate and reproduce together, at the same time that they remain protected from environmental stress. Pathogenic prokaryotes often rely on biofilm formation for infection.

Chimerism. In the case of protists, chimeric cell aggregations are made of groups of individual cells originated from genetically distinctive clones or cell-lines, e.g. multicellular motile "slugs" in social amebas, or even entire "fruiting bodies" (Fig. 2.3), can be mosaics of genetically different strains.

Cheating (in biology). A relationship among individual organisms in which some obtain benefits, in terms of survival and/or reproduction, at the expense of others' efforts and investments. Cheaters behave selfishly to maximize their own fitness. Organisms have tactics to detect/deter cheaters.

Clone - clone lines. Genetically identical groups of individuals, e.g. all the descendants of a single ameba (i.e. vegetative cycle). "Clone lines" or "clone cell-lines" refer to laboratory maintained, or wild-captured individuals or cells whose genetic identical descendants are perpetuated over time.

Epigenetic phenomena. Environmentally-induced modifications of gene expression without changes of the underlying genetic sequence.

Feral strains. Cell lines recently collected in the wild and kept under laboratory conditions that resemble the natural environment. Feral strains differ from "domesticated" cell lines, which have been extracted from nature in the past but kept in the laboratory for many decades, even centuries.

Flocculation (in biology). The assemblage of large clumps of eukaryotic cells, or "flocks," consisting of thousands of cooperating (for protection or nutrient metabolism) or reproducing individuals, e.g. yeast cells can flocculate while processing sugars during fermentation for beer production.

Green-beard effect. A metaphor to explain the genetic nature of altruistic cooperation among individuals carrying a label/tag for identity, the "green beard" (as per Dawkins 1976). The gb-effect is equivalent to the "super gene" or "recognition alleles" model (Hamilton 1963, 1964; below).

Green-beard genes: Multi-allele replicators/genes responsible for expressing reciprocal recognition traits among close genetic relatives. Green-beard genes are associated with or facilitate adaptive cooperation/altruism.

Knockout gene. An inactivated replicator/gene (i.e. via genetic engineering).

Pheromones. Molecules that, once released into the environment by organisms, influence the behavior of other organisms (i.e. conspecifics).

Pleiotropic gene. A genetic replicator responsible for multiple effects, e.g. the *tgrB1–tgrC1* alleles regulate cell-cell adhesion and slug-/fruiting-body development in social amoebas (Wang and Shaulsky 2015).

Polymorphic gene. A genetic replicator (or replicators) possessing multiple alleles that vary in sequence and expression.

Recognition alleles. The genetic mechanism of discrimination/recognition of closely (versus distantly) related conspecifics. "Recognition alleles" refers to both the 1960s theoretical proposal that organisms rely on genes to identify cooperation partners and behave altruistically toward them (as per Hamilton 1963, 1964), as well as the experimental demonstrations of genes involved in such process, e.g. *FLO*, *csA*, *tgr*, *AX4* and *QS* (this chapter).

The recognition alleles model remained a theoretical supposition for thirty years, until Keller and Ross (1998) reported —via indirect evidence— its possible existence in the red fire ant *Solenopsis invicta*. These authors described that in ant colonies Bb workers were able to distinguish BB from Bb queens via odor cues (pheromones), and with the peculiarity that Bb workers killed BB queens; a behavior induced by the allele *Gp-9^b*, which was hypothesized to be linked to a green-beard effect (Fig. 2.1a). Because the bb genotype had low viability and/or fertility (i.e. zero representation in the population), the authors were able to quantitate with confidence the proportion of queens of each of the viable *Gp-9^b* genotypes that were attacked by the workers. They found that, at age 1 (early in life) and age 2 (later in life), the workers consistently killed the BB queens (61% of queens were killed by workers of age 1, and 91% of queens were killed by workers of age 2) but never killed the Bb genotype (Fig. 2.1b). In addition, the proportion of workers of each *Gp-9^b* genotype (either BB or Bb workers) that recruited around attacked or non-attacked queens (also of each genotype, BB or Bb queens) allowed the authors to infer the possible existence of recognition alleles that functioned via a green-beard effect: BB workers had low recruitment around attacked BB queens (21% of BB workers) or non-attacked Bb queens (34% of BB workers). However, 78% of Bb workers recruited around and killed BB queens (i.e. suggesting high drive to recruit around BB queens to kill them). By contrast, 62% of Bb workers recruited around and never killed the Bb queens (i.e. suggesting usual drive to recruit around Bb queens and coexist with them; Fig. 2.1c).