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 ($_rb$) surpass the costs (c), then altruistic cooperation could evolve ($_rb - c > 0$).

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 amebas, 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.

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