Behavioral Ecology Advance Access published September 30, 2015

Behavioral Ecology

The official journal of the ISBE International Society for Behavioral Ecology

Behavioral Ecology (2015), 00(00), 1–5. doi:10.1093/beheco/arv154

Invited Ideas

Using bacteria to study consistent variation in individual behavior

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Received 31 July 2015; revised 28 August 2015; accepted 28 August 2015.

Two recent observations in behavioral biology have sparked great interest and have already yielded many novel and intriguing insights. Bacteria appear to live lives of unforeseen behavioral complexity, and the consistent behavioral variation among individual animals is often not "noise" but turns out to be a highly relevant ecological and evolutionary feature in itself. Research covering these 2 phenomena has proceeded largely in isolation, and the rich behavioral lives of bacteria have not yet been studied with consistent interindividual behavioral differences in mind. Yet, the parallels between animal and bacterial behavior that are increasingly being uncovered, as well as the particular characteristics of bacteria, point toward a new approach in the study of consistent individual variation in behavior. Using bacteria can bring fruitful opportunities to the field and allows researchers to address questions that are very difficult to pursue using animal model systems. Notwithstanding a few challenges, bacteria can provide an alternative study system that may elucidate several evolutionary and ecological aspects of consistent individual behavioral variation.

Key words: **animal, bacteria, behavior, individual, variation.**

INTRODUCTION: BACTERIA AND BEHAVIOR

The study of consistent individual variation in behavior, often referred to as animal personality, has blossomed in recent years. Individuals of the same species are often found to differ consistently in their behavior, both within and between populations. To understand the evolutionary and ecological causes and consequences of this variation, the main study subjects are, perhaps unsurprisingly, vertebrates. These, however, are not the only organisms that exhibit variation in a range of behaviors. In addition to the recent call to include more invertebrate taxa in the study of animal personality (Kralj-Fišer and Schuett 2014), it is being increasingly recognized that bacteria and plants can show suites of complicated behaviors (Crespi 2001; West et al. 2007; Karban 2008).

There are tantalizing hints that bacteria provide a tractable study system to examine behavioral variation that can be integrated with animal behavioral ecology more readily than often assumed. Recent work on the intricate social lives of bacteria shows that there is a variety of behaviors that can be investigated (Crespi 2001; West et al. 2007). Additionally, high levels of within and between strain phenotypic diversity have been noted (e.g., Darch et al. 2015). In most cases, the behavior of individual bacterial cells is pooled into the average behavior of groups known as cheaters, persisters, etc. There are, however, some historical precedents in the study of phenotypic variation in bacteria at the level of individual cells (Kelly and Rahn 1932; Powell 1955; Spudich and Koshland 1976).

BEHAVIOR IN BACTERIA AND ANIMALS

Interestingly, the focus of 2 of the earliest studies on individual bacterial phenotypic variation was growth rate (Kelly and Rahn 1932; Powell 1955). Along with the recent work that suggests a connection between bacterial behavior and growth rate or metabolism (Taylor and Zhulin 1998; Li et al. 2014), this offers a potential link with animal behavior studies that tie individual behavioral variation to growth rate (Stamps 2007) and metabolism (Careau et al. 2008; Réale et al. 2010).

In addition, there are more parallels. Environmental variability and food availability have been implicated as relevant actors in the development and maintenance of animal personality (Sih and Bell 2008; Luttbeg and Sih 2010; Wolf and Weissing 2010). Similar ideas have been proposed to understand phenotypic, and more specifically behavioral, variation among bacteria, whether the focus lies on chemotaxis (Frankel et al. 2014) or social behavior (Park et al. 2003). More generally, resource partitioning, temporal

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variation, and spatial structure all play a role in the emergence and preservation of bacterial phenotypic variation (Rainey et al. 2000).

An animal's or bacteria's environment is not composed solely out of abiotic ingredients. The presence of other organisms molds behavioral variation as well. Antagonistic interactions, such as predation and parasitism, are associated with the rise and maintenance of animal personality (Dall et al. 2004; Dingemanse and Réale 2005; Bell and Sih 2007; Barber and Dingemanse 2010; Kortet et al. 2010). In the bacterial realm, corresponding findings concerning the effects of predation (Morgan et al. 2010) and phage infection (Bohannan and Lenski 2000; Buckling and Rainey 2002; Webb et al. 2004) on phenotypic variation have surfaced.

Organisms also interact with members of their own species. The social environment formed by these interactions can impact the presence, absence, and pattern of consistent individual behavioral variation. The social niche hypothesis stipulates that, whether through choice or coercion, individuals in a population adopt a social role and, in doing so, influence the behavioral variation present (Bergmüller and Taborsky 2010). The social lives of microbes have been getting much attention lately (Crespi 2001; West et al. 2007; Xavier 2011) and behaviors such as cooperation and cheating are the focus of substantial research efforts. Through manipulating group composition, one can test the social niche hypothesis (e.g., Laskowski and Bell 2014; Laskowski and Pruitt 2014). Bacteria provide a useful system to do this. For example, introducing a mutant strain that does not contribute to the production of common goods (e.g., siderophores in *Pseudomonas* spp.) establishes a proportion of "forced" cheaters in a population. Noncheaters have been shown to partially compensate for the resulting loss of common goods (Harrison 2013). Does this compensation alter their behavior? Vice versa, does it affect the cheaters' behavior?

A specific aspect of social behavior involves group behavior. In bacteria, quorum sensing and biofilm formation are well-studied examples. Group behavior, and especially its relation to the behavior of the constituent individuals, has only recently gained traction in research covering animal behavioral variation. Most animal studies relating group and individual behavior have been done in eusocial insects and social spiders (e.g., Chapman et al. 2011; Hui and Pinter-Wollman 2014; Jandt et al. 2014; Keiser et al. 2014; Pruitt and Keiser 2014). Recently, however, phenomena such as shoaling and gregariousness have been studied in other species as well (e.g., Magnhagen and Bunnefeld 2009; Jolles et al. 2015; Planas-sitja et al. 2015). In contrast, among bacteria the group rather than the individual is the level that attracts most attention.

BACTERIAL PERSONALITY?

Variation is the bedrock of natural selection, and, as such, we expect to find it throughout life's kingdoms. Seeing behavioral variation in animals and bacteria should not be surprising. However, the mystery of "animal personality" lies in the consistent interindividual differences. To maximize fitness in the wide variety of environmental conditions that many organisms experience during their lifetime, a high degree of behavioral flexibility would be very useful. Instead, individual animals tend to display a rather limited plasticity in behavior, and the behavioral differences among individuals are often conserved across contexts (Dingemanse and Réale 2005). Do we find this in bacteria as well? If so, can we use this to explore the phenomenon in ways that are difficult or unfeasible in animal systems?

Individual phenotypic variation in bacteria has not yet received a lot of attention, despite a few historical precedents (Kelly and Rahn 1932; Powell 1955; Spudich and Koshland 1976). Nevertheless, the occurrence of phenotypic heterogeneity and behavioral plasticity in bacterial populations is increasingly being recognized (Harrison 2013; Frankel et al. 2014), as is its importance in phenomena such as quorum sensing, biofilm formation, and antibiotic resistance (for more details, see Drenkard and Ausubel 2002; Balaban et al. 2004; Cárcamo-Oyarce et al. 2015).

Despite parallels and complementary efforts, we should not be blind to the differences between animal and bacterial study systems. Animals are not bacteria. To infer that what holds for one also holds for the other is, at best, premature. A first step to explore the extent of the commonality between both is to address aspects of behavioral variation that have been studied well in one, but less in another system: group-level behavior and its relation to the behavioral variation among the constituent individuals in animals on the one hand, and consistent individual behavioral variation in bacteria on the other.

Studying behavioral variation implies selecting one, or several, behavior(s) to assess. Rather than artificially trying to develop similar assays for animals and bacteria, a more fruitful approach might be to ask which behavioral axes are most relevant for the taxon being studied and design assays that can capture variation in these axes under different ecological conditions. For example, the previously mentioned common goods production by *Pseudomonas* spp. provides an opportunity to investigate the cooperative production of a resource in different social regimes (i.e., more or less cheaters). Alternatively, cooperatively hunting bacteria such as *Myxococcus* spp. (see Crespi 2001; Morgan et al. 2010) can be used to investigate prey capture efficiency at different prey availability rates and how this affects individual and group behavior, as well as the link between both.

CHALLENGES...

To investigate consistent individual behavioral variation, bacteria can provide an alternative, complementary framework. Bacteria are quite different from the animals often used in studies of consistent individual variation in behavior, and yet, research in the past few decades has shown that several aspects of animal behavior have analogues in the bacterial realm, especially social behaviors (e.g., Crespi 2001, but see Dunny et al. 2008 for a cautionary note on interpreting and extrapolating these behaviors).

However, using bacteria to investigate individual variation in behavior is not without its challenges. A key component in the definition of animal personality is consistency across time and contexts (Dall et al. 2004; Sih et al. 2004; Dingemanse and Réale 2005). Thus, a sturdy parallel with animal behavioral variation requires some degree of consistency in the observed variation in bacterial behavior. To establish this, a specific behavior needs to be quantified on several separate occasions. The short generation time of most individual bacterial cells renders this challenging. Alternatively, if one chooses the genotype as marker of individuality (Janzen 1977), this brevity of generation time can lead to a rapid accumulation of mutations, causing the starting genotype and the genotype tested on subsequent times to diverge. It is difficult and somewhat arbitrary to determine when this results in comparing 2 distinct individuals rather than 1 individual over time.

Also, studying individual behavioral variation in wild populations is challenging, even in animal systems (Dingemanse and Réale 2005). The relative lack of knowledge concerning, and difficulty of observing, natural microbial populations makes this even harder in bacteria.

...AND OPPORTUNITIES

However, bacteria also present opportunities for behavioral ecologists, in some ways mirroring and extending the advantages of studying individual behavioral variation in invertebrates (see Kralj-Fišer and Schuett 2014) (Figure 1). By taking advantage of the peculiarities of bacterial study systems, questions concerning the evolutionary and ecological dynamics of consistent individual behavioral variation that are hard to tackle in animal study systems can be addressed.

The short generation time provides a prospect to assay behavior across a number of generations that is rarely feasible in animals. Relatedly, the rapid accumulation of mutations could, if certain mutations can be coupled to behavioral differentiation, elucidate some of the genetic foundations of behavioral variation (for links between molecular mechanisms and phenotypic variation in bacteria, see Korobkova et al. 2004; Smits et al. 2006). These aspects of bacterial systems could be very helpful in addressing questions concerning the fitness consequences of behavioral variation. One could, for example, start with a bacterial population composed out of bacteria of similar behavioral type and observe whether or not variation among individuals increases in subsequent generations. If so, this provides a strong indication of the adaptive value (or at least of the absence of negative fitness consequences) of behavioral variation within a population, even when the individuals are exposed to identical conditions (see van Vliet and Ackermann 2015). This also provides the opportunity to establish whether or not a plateau is reached in the amount of behavioral variation present in a population, and, if so, how long it takes to achieve this plateau and how stable it is. The genetic tools and genomic information available for many bacteria offer the additional chance to investigate and manipulate the genetic roots of the observed changes.

Additionally, using bacteria allows researchers to establish and study populations of a size that is unachievable in animal systems. Observing the emergence and ultimate fate of rare behavioral types is more likely to occur in larger populations, and being able to witness evolution in real time in large populations brings questions concerning the evolutionary dynamics of individual behavioral variation into view. Such large populations enable researchers to, for example, take several behaviorally similar and dissimilar, or behaviorally common and rare, samples of a population and introduce these to a new environment. By letting these offshoots develop, the phenomenon of behavioral founder effects can be studied in greater detail than possible in animal systems. Is the duration of these effects different for different behavioral types? Are the effects identical for behaviorally similar founders? How do they influence the behavioral variation present after many generations?

Furthermore, the ability to establish a substantial number of clonal individuals can circumvent the challenge of studying developmental influences on individual behavioral variation in animals. After all, each individual animal has a unique developmental history (see the replicate individual design in Stamps and Groothuis 2010). Studying the ontogeny of personality differences can benefit greatly from having many clonal individuals at one's disposal. The ability to assess the behavioral evolution of individuals that possess the same genes and are exposed to the same tightly controlled environment allows researchers to address questions concerning both the behavioral development of individuals and of the behavioral variation in populations. Starting with many replicate individuals and allowing these to develop and reproduce asexually in identical and different environments is an excellent starting point to address questions such as: Do clones raised in identical environments undergo the exact same behavioral development (i.e., how significant is the contribution of "molecular noise" to nongenetic individuality in homogenous conditions, see Korobkova et al. 2004)? Do clonal founder cells give rise to populations that display similar behavioral variation, if any, among their constituents? At which point in (evolutionary) time, does behavioral divergence start? The same questions can be asked for clones in different environments, providing a way to way separate the relative genetic and environmental contributions to the evolution of behavioral variation. The degree of environmental control and amount of clonal individuals required make bacteria a particularly suited study system to tackle these issues.

Finally, although studying bacterial behavior in natural populations is difficult, the degree of environmental control possible in a lab setting is very high, which can reduce ecosystem complexity to a point where singling out and manipulating specific contributing factors becomes a viable option (Rainey et al. 2000). This means that in the previous experimental proposals the ecological context

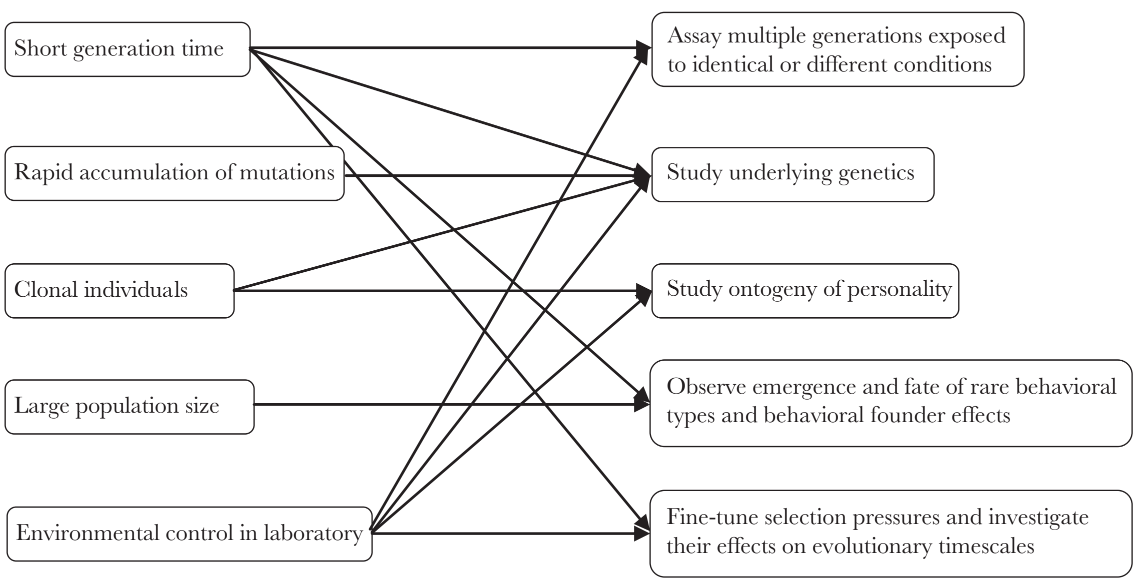

Figure 1
The peculiarities of bacterial study systems (left) can provide unique opportunities (right) for the study of individual behavioral variation.

can be manipulated and controlled to a degree (and for evolutionary timescales) seldom achievable in animal study systems. As such, the context dependency of the ecological and evolutionary development and maintenance of consistent individual variation in behavior can in itself become a major and highly controllable topic of study.

Additionally, taking the study of individual behavioral variation beyond the paradigm study system, that is, animals (and specifically vertebrates), is useful to discern whether or not there are general evolutionary and ecological principles at work (see also Kralj-Fišer and Schuett 2014). Are certain influences relevant only in some taxa but not in others? What is the role of organismal complexity, if any? How do specific characteristics of species affect the origin, maintenance, and evolution of behavioral variation? These are the questions that are best addressed when data are available for taxa spread across the web of life.

CONCLUSION

Exploring novel study systems to investigate consistent variation in individual behavior can prove beneficial and allows a broad perspective on this perplexing phenomenon. Bacteria are an underexplored study system in this field, but their short generation time, the genetic and environmental control, and the possibility of establishing large and clonal populations are all aspects of their biology that can help researchers address a suite of exciting questions.

FUNDING

G.D.W. is funded by a PhD studentship from the School of Life Sciences at the University of Nottingham. J.P.S. is funded by a BBSRC fellowship. B.B.C. is funded by a Research Fellowship from the School of Life Sciences at the University of Nottingham.

We would like to thank S. Diggle and F. Harrison for thoughts from the bacterial side of the equation. We also thank the 2 anonymous reviewers whose insightful and encouraging comments greatly improved this article.

Editor-in-Chief: Leigh Simmons

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