Group phenotypic composition shapes emergent network structures and selection regimes in *Bolitotherus cornutus*

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A Dissertation presented to the Graduate Faculty
of the University of Virginia in Candidacy for the Degree of
Doctor of Philosophy

Department of Biology

University of Virginia
May 2022

ACKNOWLEDGEMENTS

This research was made possible by funding from a National Science Foundation Graduate Research Fellowship, the Research Experience for Undergraduates program at Mountain Lake Biological Station, NSF grants to Butch Brodie and Vince Formica, the Margaret Walton and Horton H. Hobbs Scholarships, the UVA Department of Biology, the UVA Double Hoo Award, and a professional development fund from the UVA Graduate School of Arts and Sciences. This means my work was funded by taxpayers, with assistance from generous donors. Thank you all.

None of this work would have been possible without my mentors. Butch Brodie taught me about both big picture ideas and the day-to-day process of science. Whenever I asked him to go along with something, he threw his full support behind me—even if he wasn't confident it would work. That is true not only of my experiments but also of fundamental issues of how we form our communities and treat one another, and I will always be grateful for his willingness to listen and learn. And of course he kept me well fed on paella and gave me opportunities to keep my first aid skills sharp. Vince Formica has been my unofficial second advisor, in addition to being the one who first made me fall in love with evolution. Vince, thank you for your listening ear and endless willingness to go down whatever weird little rabbit hole I found myself in. You have an incredible gift of always making me feel better, no matter what the problem is. My committee members Robert Cox, Laura Galloway, Alan Bergland, and Dave Carr gave me thoughtful feedback at every stage of this process and pushed me to be a better, more well-rounded scientist. And Idelle Cooper was always willing to give me advice and encouragement, long after I wasn't her graduate student any more.

Thank you to everyone who makes the Brodie Lab such a fun, supportive, collaborative place to work. Liza Mitchem generously helped build the beetlearies and offered statistical advice. Eileen Butterfield, Savanna Cabrera, and Hannah Donald put in the hard work to build and maintain the systems I relied on for basically everything in the field. And of course Robin Costello was my partner in all of this, the founder and co-PI of the beetlearies. We spent thousands of hours working together on every aspect of this project. Robin, not only could I not have done this without you, it's hard to even say where your work stops and mine begins. I learned so much from you, and I could not have asked for a better collaborator.

I have been so lucky to work with extraordinary undergraduates who helped me remember the joy of studying nature. Livi Baker, Olivia Shaffer, Matthew Armstrong, and Alejandro Medina-Valencia all brought their unique personalities to their projects and taught me so much. Chapter One of this dissertation was a collaboration with Livi Baker and would not have been possible without her. I also benefitted from the hard work that the many, many students on Beetle Crew have done over the years to collect data and explore new ideas, which laid the foundations for what I have done. In particular, Chapters Two and Three of this dissertation build upon a project led by Vitor Dos Anjos.

I came to UVA to be able to work at Mountain Lake Biological Station, and the start of every summer felt like coming home. Thank you to everyone who helps make that community what it is. Some of my fondest memories will always be there with Stephanie Carrera, Hannah Marti, Chloe Lash, Hannah Donald, and so many other weird, wonderful scientists.

Thank you to the station staff, past and present, for all you do. The kitchen staff, cleaning crew, and Rhonda Ruff worked tirelessly in the background to keep the station running so that we could focus on research. Jaime Jones let me make a mess in the aquatics lab with the fungus sprinklers every summer, and also made accommodations for my mental health that made a profound difference in my quality of life at the station. Thank you to Eric Nagy for your kindness, diligence, and of course for hosting the first annual Mountain Lake Film Festival. Thank you to Tom Mc Namara for not only keeping the whole place standing but also taking the time to teach me any skill I wanted, cook me gourmet meals, prepare me to be a first responder, and talk openly about trauma. Henry and Becky Wilbur taught me to value natural history and always remember that there is more to learn. Thank you, Paw Paw.

So many other people in the Biology department have supported me over the past five years. To name a few: Dave Kittlesen, Joanne Chaplin, and Kay Christopher made TAing easy and fun. Jessamyn Manson provided invaluable advice and feedback on my teaching. Megan Champion was always willing to help me find an answer or solve a problem, and Sherrie Jones made me laugh every time I walked into the office.

I have found community in so many places: GRIT, VISAS, UVA Survivors, Organizing 4 Survivors, and more. I am especially grateful for my fellow members of the United Campus Workers of UVA, who doggedly insist on solidarity and who make me believe that progress is possible. And during the pandemic the Blue Ridge Mountain Rescue Group gave me the sense of purpose (not to mention regular human contact) that kept me sane.

On the subject of sanity, thank you to my therapist at the UVA Women's Center, who has supported me for four years. We met during one of the lowest points of my life, and I owe so much of my growth and happiness since then to our conversations.

I cannot possibly thank all the friends who have supported me, but some I can't leave out. Rebecca, I was unfathomably lucky to end up with you as a roommate and a friend. There is no one else I would rather have lived with for the past five years. Thank you for all the rides, bread, laughs, shared meals, and movie nights in the queer little home we made together. Stephanie, I am so glad we were assigned to share a tiny little cabin room together that first summer at Mountain Lake. You have become one of the great loves of my life, and it has been incredible to watch you grow. Thank you to Peter for our weekly book club talks, which have helped me wrestle with some of the big questions about myself and the world. And a shout out to all of my friends who stepped off the default path to go after what you want, especially those who have gone back to school: Tom, Esther, Stephanie, and Gabrielle, I am proud of you all.

G, thank you for being my sister, my friend, my co-signer on terrifying legal documents, and my first call for anything from medical emergencies to minor curiosity questions. Thank you also for accepting that I will usually ignore your advice and just try to solve every problem with a knife. We have done amazing things, both individually and together, and ours will always be the most important relationship in my life. You are such a competent, thoughtful, resourceful, and deeply ethical person. Dad would be proud (if he were here, which he would be the first to say he is not).

Jamie has been my partner in this voluntarily constituted federation of ours, which has continued to function through six years of living apart. You have been an endless source of peace, grounding me and reminding me of what I need to hear when I forget it. I love you.

Thank you to all the teachers, writers, artists, and activists who have guided and inspired me. Special thanks to Tamora Pierce, who not only gave me a role model but also once called me extraordinary over a plate of scalloped potatoes. After that, the rest of the PhD was easy.

Lastly, thank you to the beetles, who have never let us down.

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INTRODUCTION

A social network is the set of interactions or associations between all individuals in a population (Farine and Whitehead 2015). Networks can be quantified at the level of the individual, the sub-group, or the population and are thought to be under both individual and multi-level selection. Across a wide range of taxa, the position that an individual occupies within the network of its population is under natural or sexual selection (Silk et al. 2003, 2009; McDonald 2007; Ryder et al. 2008; Cameron et al. 2009; Frère et al. 2010; Schülke et al. 2010; Stanton and Mann 2012; Wey and Blumstein 2012; Brent et al. 2013; Gilby et al. 2013; Wey et al. 2013; Bar Ziv et al. 2016; Cheney et al. 2016; Lehmann et al. 2016; Blumstein et al. 2018; Bond et al. 2021). Although the mechanisms of this selection are not yet clearly established in any system, network position modulates individuals' exposure to grooming, social information, parasites, and disease (Flack et al. 2006; Drewe 2010; Claidière et al. 2013; Dey et al. 2013; VanderWaal et al. 2014; Aplin et al. 2015; Carter et al. 2020). Group social structures themselves are also thought to be adaptive, with aspects of group organization predicting the fitness of group members in some insects (Gordon 1996; Pinter-Wollman et al. 2014; Reia and Fontanari 2017; Costello 2020).

Increasing attention is being paid to social networks as both potential products and drivers of evolution (Fisher and McAdam 2017; Bailey and Moore 2018; Montiglio et al. 2018), but there are still many unanswered questions about social network phenotypes. In particular, it remains unclear 1) to what extent variation in social network traits are explained by individual as opposed to partner identity, 2) why selection gradients on these traits vary among groups with a species, 3) what drives variation in network structures among groups, and 4) how individual and group network traits are related (Cantor et al. 2021).

My dissertation work contributes to answering each of these questions. This research was performed in captive populations of the forked fungus beetle *Bolitotherus cornutus* (Panzer 1794; *Opatrum bifurcum*, Fabricius 1798, 1801). *Bolitotherus cornutus* is a cryptic tenebrionid beetle which lives on the bracket fungi decomposing fallen logs in the forests of eastern North America. During the summer adults feed, mate, and lay eggs on the surfaces of the host fungus, and larvae develop inside the brackets (Liles 1956; Pace 1967). Their social interactions take place on these "behavioral arenas," and both male and female fitness can be estimated from behavioral observations (Conner 1988). Beetles live clustered into subpopulations, each consisting of the residents of a single log. There is a long history of research on the metapopulation of forked fungus beetles in the woods around the University of Virginia's Mountain Lake Biological Station (Brown and Bartalon 1986; Whitlock 1992; Ludwig 2008; Wood et al. 2018; Formica et al. 2020).

Social behaviors and their fitness consequences have been well characterized in this system. Social network position is under sexual selection (Formica et al. 2012), although the magnitude and even sign of that selection varies across the metapopulation (Formica et al. 2020). Work I contributed to during my undergraduate studies found that social network position is a repeatable property of individuals over the timescale of a few weeks when they remain with the same social partners, even after a disturbance (Formica et al. 2017). The physical distribution of resources in the environment impacts individual social network position, although only slightly (Costello et al. 2022). Group-level aspects of network structure are also under multi-level selection (Costello 2020).

My dissertation research used an experimental approach to allow for strong tests of the effects of group phenotypic composition (Farine et al. 2015) on individual and group network traits, fitness, and selection regimes. I performed two large-scale manipulations of group composition in mesocosm populations of forked fungus beetles on the grounds of Mountain Lake Biological Station in Giles County, Virginia. These mesocosms allow for one demographic variable to be changed while controlling for others, including the group size effects which often confound attempts to compare social networks (Farine and Aplin 2019). They are also replicated, which makes them powerful tools for answering current questions in social network research (Krause et al. 2010; Smith et al. 2019). This experimental system, established in collaboration with Dr. Robin Costello, has already produced insights into the social consequences of resource distribution (Costello 2020; Costello et al. In press) and the factors contributing patterns of phenotypic assortment which shape the response to selection (Brodie et al. 2021).

In Chapter One, I report the results of the first of my experiments. In the summer of 2019, I assigned beetles to one of two treatments based on their past social behavior. I ask whether individuals maintain their social tendencies when placed into new groups or alter their behavior based on the individuals around them. I find that individuals maintain both their number of partners and number of interactions in a new context, adding further evidence that sociability is a personality trait (Gartland et al. 2021) and suggesting that at least some variation in individual social network position is explained by individual rather than partner identity. Additionally, I show that the composition of social personalities within a population dramatically alters overall network structure. This has two significant implications. First, it suggests that the personalities of group members could affect the way that information and disease move through groups. Second, it demonstrates a causal link between individual personality and network structure that could help explain how social networks evolve. If individual social network position has a genetic basis, as has been demonstrated in other systems (Fowler et al. 2009; Lea et al. 2010; Brent et al.

2013; Wice and Saltz 2021), and differences in individual sociality contribute to network structures, these structures would be expected to respond to selection (whether individual or multi-level).

My second experiment, reported in my second and third chapters, used a similar design but manipulated a different axis of variation. In the summer of 2020, I created groups that differed in their age composition. I assessed the consequences of variation in age at multiple levels of social organization for social structures, fitness, and selection. In Chapter Two, I describe how age creates variation in social behavior within individuals over time, among individuals, and among groups. Unlike personality, the composition of ages within a group does not affect the number of social interactions within a population, but does affect how those interactions are distributed across all possible pairs. I highlight the age structure of populations as a source of temporal and spatial variation in both individual network position and network structure.

In Chapter Three, I investigate how age at multiple levels of organization affects fitness. I show that age is associated with fitness across three different levels of organization: the individual, social group, and population. Older beetles of both sexes have higher reproductive success than newly emerged tenerals; males, but not females, pay a fitness cost to interacting with older male partners; and both sexes have lower fitness in groups of old members. In females, sexual selection on social network position depends on the age structure of the population. My results suggest that differences in the age of the social environment, within or among populations, are a source of variation in both fitness and selection pressures. I highlight that population age structure may be another demographic factor, like operational sex ratio or density, that determines the strength of sexual selection.

Taken together, these findings contribute to our understanding of the basis of individual and group differences in social traits, as well as the relationship between them. They also propose a new perspective on age. Individuals of different ages both experience and affect their populations differently. Age has now been found to correlate with almost every fitness-relevant trait in this system (Figure 1). Note that only body and horn size are not conclusively linked with age; one early study suggested that differential survival is associated with morphology(Conner 1988), but the results may conflated emigration with death and should be repeated. With this exception, age emerges as a potential source of correlations between traits and variance in the opportunity for selection. Overall, my results point to age as an understudied variable linking metapopulation processes, social structures, and selection regimes.

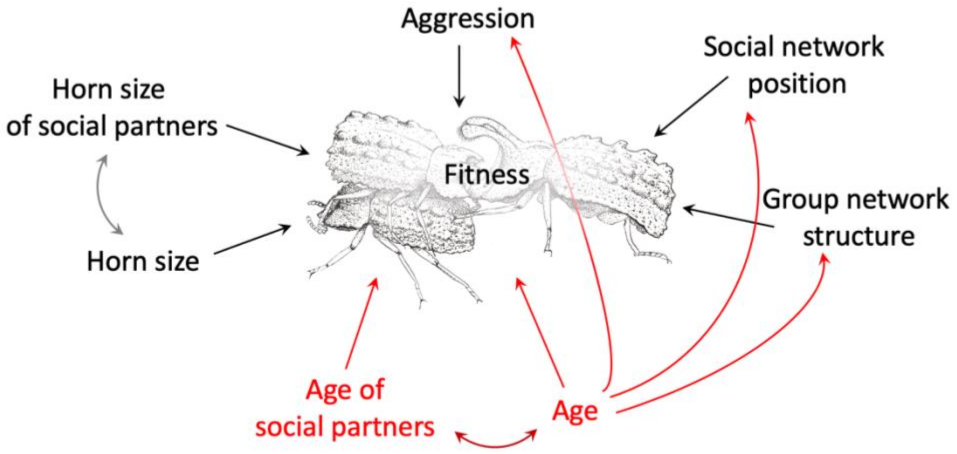

Figure 1. Our current understanding of factors influencing reproductive components of fitness in the forked fungus beetle, with effects of age highlighted in red. All age effects other than that on aggression (described in Mitchem et al. Submitted) come from this dissertation work.

My work has also built the foundations for future research. Captive populations and mesocosms can be used for a range of powerful experiments. My first chapter raises questions about the evolvability of social networks, and future work could make use of my network datasets and stored genetic samples to measure direct and indirect genetic effects on social network position. My second chapter's findings of longitudinal change in social behavior raise the questions of 1) to what extent social behavior changes with age because of accumulated experiences versus intrinsic changes, and 2) whether increasing social selectivity with age is a widespread phenomenon across long-lived taxa. My third chapter suggests that age structure is a previously overlooked source of variation in sexual selection regimes. Future work could test whether this is the case in natural populations.

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CHAPTER ONE: Group composition of individual personalities alters social network structure in experimental populations of forked fungus beetles[1]

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[1] Formatted as a coauthored manuscript, published in *Biology Letters:* Cook, P. A., O. M. Baker, R. A. Costello, V. A. Formica, and E. D. Brodie III. doi: 10.1098/rsbl.2021.0509

Abstract

Social network structure is a critical group character that mediates the flow of information, pathogens, and resources among individuals in a population, yet little is known about what shapes social structures. In this study, we experimentally tested whether social network structure depends on the personalities of individual group members. Replicate groups of forked fungus beetles (*Bolitotherus cornutus*) were engineered to include only members previously assessed as either more social or less social. We found that individuals expressed consistent personalities across social contexts, exhibiting repeatable numbers of interactions and numbers of partners. Groups composed of more social individuals formed networks with higher interaction rates, higher tie density, higher global clustering, and shorter average shortest paths than those composed of less social individuals. We highlight group composition of personalities as a source of variance in group traits and a potential mechanism by which networks could evolve.

Introduction

Animal social networks describe patterns of interaction among individuals. The position an individual occupies within a network modulates its exposure to information, parasites, pathogens, grooming, and other consequences of sociality (Flack et al. 2006; Drewe 2010; Claidière et al. 2013; Dey et al. 2013; VanderWaal et al. 2014a; Aplin et al. 2015a; Carter et al. 2020). Over the last two decades, research across a range of taxa has confirmed that individual social network position has fitness consequences (Silk et al. 2003; Ryder et al. 2008; 2009; Oh and Badyaev 2010; Schülke et al. 2010; Barocas et al. 2011a; Gilby et al. 2013; Wey et al. 2013; Bar Ziv et al. 2016; Cheney et al. 2016; Yang et al. 2017; Formica et al. 2020; Bond et al. 2021). Position within a network is known to be a repeatable trait both within the same group (Jacoby et al. 2014; Krause et al. 2016; Kulahci et al. 2018; O’Brien et al. 2018; Jones et al. 2019) and in some species across social contexts (Blaszczyk 2018; Strickland and Frère 2018; Plaza et al. 2020). Revealing the sources of variation in individual network position has led to a deeper understanding of how and when such traits are expected to evolve (Pinter-Wollman et al. 2014; Cantor et al. 2021).

Much less is known about the causes and consequences of variation in network structure at the group level (Ilany 2020). Group social network structures are emergent traits generated by the interactions of all group members. These structures affect ecological processes such as the transmission of information and disease, as well as a number of other demographic and evolutionary phenomena (reviewed in Kurvers et al. 2014; Bailey and Moore 2018; Cantor et al. 2021). It is often suggested that network structure can be adaptive (Gordon 1996; Nandi et al. 2014; Pinter-Wollman et al. 2014; Reia and Fontanari 2017), although only a few studies have demonstrated this empirically (Barocas et al. 2011a; Wey et al. 2013; Royle et al. 2012; Solomon-Lane et al. 2015; Stroeymeyt et al. 2018; Costello 2020). Network structures are repeatable across years or environments in some species (Godfrey et al. 2013; Shizuka et al. 2014; Wilson et al. 2014, 2015; Aplin et al. 2015c; Blaszczyk 2018), adding further evidence that they are non-random.

One possible source of variation in network structures is the composition of individual personalities within groups. If individuals differ and maintain these differences across social contexts, group-level traits may depend on the composition of the group. Behavioural types of group members are known to affect other emergent traits, including group activity (Sih and Watters 2005), foraging success (Laskowski and Bell 2014; Jolles et al. 2017), prey capture (Keiser and Pruitt 2014; Pinter-Wollman et al. 2017), mating system (Montiglio et al. 2017), and collective offspring care (Hillesheim et al. 1989). There is evidence that group composition is a source of variation in social network structure, but this work has focused on traits that can be phenotyped in isolation (Pike et al. 2008; Dakin et al. 2021).

No studies have investigated how shifting the frequencies of social personalities within a group impacts network structure, despite growing interest in sociality as a personality trait (Gartland et al. 2021). The inherently multi-level nature of networks creates a clear link between individual behaviour and overall structure—a group whose members interact frequently and widely must have a densely connected network, while a group whose members interact rarely with few partners will have a sparse network. What is not known is whether individual behavioural types are consistent properties of individuals maintained in new social contexts, such that altering their frequencies will create variation in networks (Krause et al. 2010). Alternatively, if individuals alter their behaviour to fill social niches (Bergmüller and Taborsky 2010; Montiglio et al. 2013), network structure may effectively be regulated and will not differ with group composition. In other words, we are asking whether individual behavior is a predictor of social structures or solely an outcome of them. Insight into how group traits arise and how they can change requires understanding how the traits of individuals affect the group as a whole.

We experimentally manipulated replicate populations of the forked fungus beetle *Bolitotherus cornutus* to test whether individuals have repeatable social personalities and whether changing the distribution of these social personalities within a group alters network structure. Using the power of replicate experimental networks (Krause et al. 2010; Smith et al. 2019), we were able to control the environmental and demographic variables that have hindered past inference about group differences (Farine and Aplin 2019), engineering populations that differed only in the social tendencies of their members. We assayed the level of sociality of each individual in initial populations, and then created experimental populations composed of either highly social or less social individuals. This approach allowed us to ask whether variation in social behaviour is a personality trait maintained in a new context and how it affects the overall architecture of interactions.

Materials and Methods

Study System

Bolitotherus cornutus is a tenebrionid beetle found on bracket fungi on rotting logs in the forests of eastern North America. Beetles within a forest are structured into subpopulations consisting of beetles living on a single log, where adults feed, interact, and oviposit on the fungus (Liles 1956; Pace 1967a). Social interactions are easily observed on the surface of the log and brackets. Past work has found that multiple metrics of social network position are repeatably expressed by *B. cornutus* over a few weeks, even after disturbance, when individuals remain in the same social groups (Formica et al. 2017). These metrics of individual position in non-mating networks are under strong but variable selection (2020, 2012). Group-level network traits are also under selection in this species; a recent multi-level selection study found that females have lower reproductive success in populations with higher social tie density (Costello 2020).

Experimental Design

We built ten large screen enclosures (Figure S1A) in the forest around Mountain Lake Biological Station (37°22'37.0"N, 80°31'17.5"W), each containing a shelf holding eighteen bags of hardwood sawdust inoculated with a lab-grown strain of the *B. cornutus* host fungus

Ganoderma tsugae. The shelves served as artificial logs, studded with clumps of fungus brackets of the same size and genotype (Figure S1B).

We created ten initial populations of thirty adult *B. cornutus* (Figure S1C) to be placed into the enclosures in June of 2019. These beetles had been held in isolation for a month prior. All individuals were identifiable by unique three-character codes printed on florescent paper and attached to their elytra with a UV-cured acrylic glue (Tuffleye Wet-A-Hook Technologies, San Antonio, TX). We measured the body size of each beetle from an image taken on a flatbed scanner (Epson Perfection V600 Photo) using ImageJ (Abramoff et al. 2003). We then assembled ten populations of 15 males and 15 females each that did not differ in average body size ($F_{9,289} = 0.06$, $p = 1$). Individuals were placed onto the artificial log haphazardly and allowed to acclimatize to their new environment for 36 hours before behavioural observation.

We phenotyped each individual's level of sociality in the initial populations. Our study system allows us to perform scan sampling of dyadic interactions, which is preferable for animal network studies because it maximizes the number of edges that can be observed and increases the robustness of the network to sampling biases (Altmann 1974; Whitehead and Dufault 1999; Davis et al. 2018). We surveyed all visible beetles three times a day for eight days. Social interactions were defined as individuals being in close proximity (within 5cm) of one another (Formica et al. 2012). The total number of interactions observed over the 24 surveys was tallied for each beetle as a measure of sociality. We then collected all beetles and held them in isolation for five days, which is sufficient time for social networks to "reset" (Formica et al. 2017a).

We reassigned individuals to new experimental populations on the basis of their observed number of social interactions, creating five populations of previously highly social beetles (mean prior interactions = 10, sd = 3) and five of previously less social beetles (mean prior interactions

= 4, sd = 2) (Figure S3). Population assignment was controlled so that mean body size did not differ among populations ($F_{9,287}$ = 1.19, p = 0.30) and the number of beetles placed together that had previously interacted was minimized. After 36 hours, we surveyed social interactions for eight days using the exact methods described above. See Supplemental Materials for additional details on experimental design and surveying.

Beetles that died during the experiment (N = 4) were removed from the networks. Observations of individuals that could not confidently be identified were excluded, but interactions with these individuals were still counted in their partner's social phenotypes. Our dataset consists of 10 initial networks (comprised of 1,041 interactions among 297 beetles; Fig. S2) and 10 experimental networks (1,341 interactions among 296 beetles; Fig. 1).

Statistical Analysis

We first asked whether two measures of sociality, number of interactions and number of partners, were repeatable from the initial social environment to the experimentally manipulated context. Repeatability is measured as the proportion of trait variance attributable to differences among individuals (Falconer and Mackay 1996). We extracted the within- and among- individual variance components from a linear mixed model with one random effect, individual identity (Nakagawa and Schielzeth 2010). Gaussian models were run in the R package MCMCglmm - (Hadfield 2010) with uninformative priors for all parameters for 500,000 iterations, discarding the first 5000 as burn-in and using a thinning interval of 100. Variable traces were stationary and did not display autocorrelation, and chains converged well (Gelman-Rubin criterion = 1 for all models). We also compared the observed repeatabilities to those calculated from 1000 permutated datasets in which the identities of nodes in the second half of the experiment were randomized.

We then asked whether social network structures differed based on population composition. We constructed 20 undirected, weighted social networks using the simple ratio index, which quantifies the strength of interaction between each dyad from 0 to 1 (Ginsberg and Young 1992). Our 24 possible observations were sufficient to estimate variance in how often dyads interact with high precision (Whitehead 2008). We then compared four group-level network metrics between treatments: interaction rate, tie density, global clustering coefficient, and length of the average shortest path between pairs. These four metrics capture different aspects of how "connected" networks are. They respectively quantify how many interactions occur per day, how many of the possible pairs are connected, how many of the possible triads are closed, and how closely linked any given pair is. Network-level metrics can be compared with conventional statistical methods when the networks are of equal size and sampled with the same methods (James et al. 2009; Croft et al. 2011; Farine and Carter 2021); see the Supplemental Materials for further details on how our design minimized differences among replicates. We used one-sided tests to test whether the populations of previously more social beetles had more interactions, higher tie density, higher clustering, and lower average shortest path lengths, as we would expect if these individuals remained highly social. All analyses were performed in R version 4.0.2 (R Core Team 2021). Weighted clustering and shortest path lengths were calculated in tnet (Opsahl 2009) and networks were visualized using igraph.

Results

We found that individual sociality was consistent; beetles maintained their level of social interaction in experimental populations with new partners. Both number of interactions and number of social partners, or network degree, were repeatable (interactions: r = 0.34, 95% CrI 0.23-0.43; partners: r = 0.39, 95% CrI 0.32-0.50). Both observed repeatabilities were significantly greater than those calculated from permuted datasets, whether we shuffled across or within treatments (interactions: $P < 0.001$, partners: $P < 0.001$).

Social network structure depended on the individual social traits within the group (Figure 1). Networks composed of beetles who had been highly social in their previous social context had more interactions per day (mean difference = 9.6, 95% CI = 3.7-15.6, $P < 0.01$), a higher density of ties (mean = 0.09, 95% CI = 0.05 - 0.14, $P < 0.01$), higher global clustering (mean = 0.12, 95% CI = 0.04-0.20, $P < 0.01$), and shorter average path lengths (mean = -0.44, 95% CI = -0.67 - -0.18, $P = 0.002$) than those composed of previously less social beetles (Figure 2).

Discussion

We found that group social networks differed dramatically depending on the personalities of their members. By experimentally engineering groups comprised of individuals with divergent levels of sociality, we showed that assemblages of highly social individuals interacted frequently and broadly, created densely connected networks with high clustering and short network distances between individuals. Conversely, groups composed of individuals who were less social in previous networks exhibited sparse networks with fewer closed triads and long paths. Individual beetles expressed consistent social tendencies even in a new social environment. These results suggest individuals in this non-eusocial insect system have social personalities, that these personalities shape group social structures, and that any process that causes the composition of personalities to vary among groups—such as a relationship between sociality and dispersal (Cote and Clobert 2007; Cote et al. 2010)—could produce variation in group structure.

Two measures of sociality, number of interactions and number of partners, had repeatability values comparable to those of other behavioral traits in this species and others (Bell et al. 2009;

Formica et al. 2017a; Mitchem et al. 2019). Our results add to an increasing body of work on sociability as a personality trait (Gartland et al. 2021). From a network perspective, these results suggest that variation in individual behavior is a predictor of social structures, but we note that there is still room for social environments to shape individual behavior and potentially create feedbacks (Cantor et al. 2021).

The differences in network structure generated by group composition may have implications for the transmission of information (Allen et al. 2013; Aplin et al. 2015a) and pathogens (Keeling and Eames 2005) through populations. One notable result is that no completely isolated nodes were found in the groups of more social individuals. Isolated individuals have lower exposure to aggression and diseases transmitted through contact, but also fewer interactions with potential mates. These impacts illustrate that both the benefits and costs of social interaction experienced by individuals will depend on the personalities of the conspecifics around them. Future work could test whether the composition of personalities within a group affects processes such as the rate of disease spread.

Variation in group composition can produce variation in group-level characters, which can in turn impact individual fitness (Farine et al. 2015; Cantor et al. 2021). *B. cornutus* females in populations with higher tie densities lay fewer eggs than those in sparsely connected populations (Costello 2020). Our results show that tie density can be impacted by the personality composition of the group, meaning that one source of variance in female fitness could be the personalities of her fellow group members. Females could therefore increase their fitness by avoiding groups of highly social individuals.

A field study found that selection on social network position was highly variable among populations of *B. cornutus*. Selection on individual strength, a metric combining the two behaviours measured in this study, trended more positively in populations with higher tie density (Formica et al. 2020). Further data are needed to confirm whether this relationship is causal. But if network structure alters patterns of selection, populations composed of individuals with more social personalities might create environments in which being social is favored, potentially creating feedback loops driving the evolution of social personality types (Farine et al. 2015).

Our results suggest that network structures could evolve through changing group composition across generations. In the absence of a group-level method of replication, the evolution of a group trait can be understood as the evolution of underlying individual traits (Okasha 2014; Sueur et al. 2019). A recent meta-analysis found that affiliative social behaviours were moderately heritable (Dochtermann et al. 2019), a few studies have found a genetic basis of individual social network position (Fowler et al. 2009; Lea et al. 2010; Brent et al. 2013; Wice and Saltz 2021), and indirect genetic pathways can provide additional heritable variation (Moore et al. 1997; Wolf et al. 1998; McGlothlin and Brodie III 2009; Fisher and McAdam 2017). Therefore, social personality types can change in frequency in response to selection. The evolution of social behaviors, whose expression in one individual may alter their expression in its partners, may be especially rapid (Moore et al. 1997; Bleakley and Brodie III 2009; Wilson et al. 2011; Franz et al. 2015; Santostefano et al. 2016; Brask et al. 2019). We have shown that shifting the composition of individual traits in a group changes group traits within a generation. This experimental result demonstrates a causal link between individual personality and group phenotypes that could help explain how the social structures of animal societies evolve.

Data Accessibility

Datasets of individual and group metrics, along with the code for statistical analyses, are available on the Dryad Digital Repository: https://datadryad.org/stash/share/Szl-d2iKjgOZCegLZ_fUesdICeFAZpIyGkXcq6J3HnM.

Acknowledgements

We thank Eileen Butterfield, Liza Mitchem, Malcolm Augat, Catherine Debban, and Fisher Brodie for construction; Hannah Donald, Savanna Cabrera, Olivia Shaffer, and Molly Sayles for data collection; Liza Mitchem for statistical advice; Henry Wilbur for feedback; and the staff of Mountain Lake Biological Station for their support.

Funding Statement

Funding for this work was provided by an NSF GRF to P.A.C., a University of Virginia award to P.A.C. and O.M.B., NSF IOS-1355029 to V.A.F. and IOS-1355003 and DEB-1911485 to E.D.B.III, and NSF REU grant 1461169 to Mountain Lake Biological Station.

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FIGURES

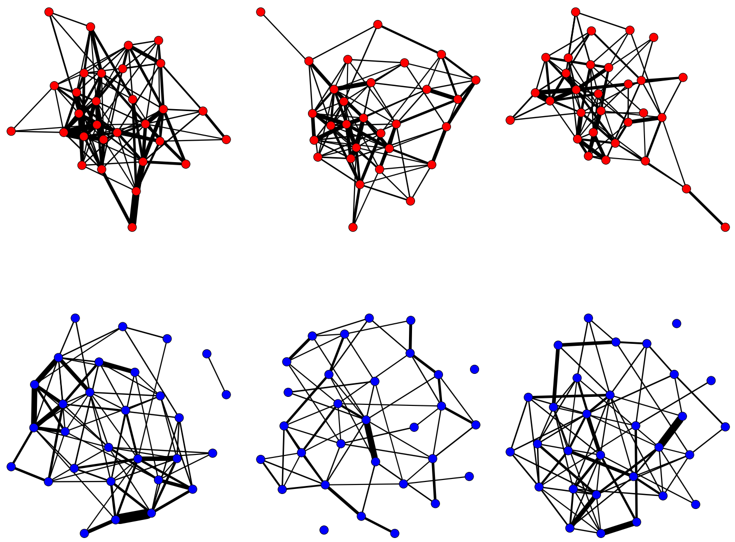
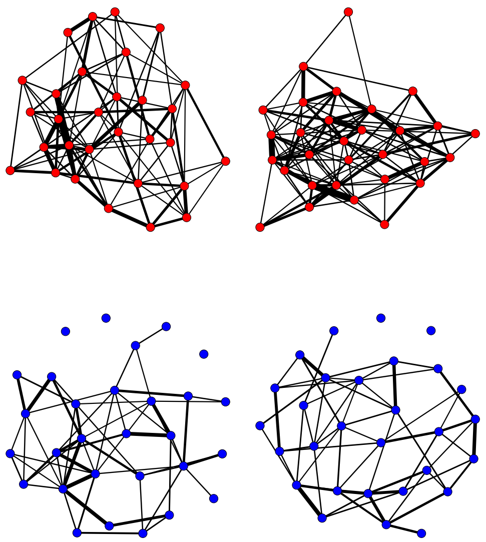

Figure 1. Networks from the ten experimental populations. Networks composed of highly social individuals (red points) differ in number, thickness, and distribution of ties from those networks composed of less social individuals (blue points). Line thickness is proportional to the simple ratio index, and nodes are plotted using an algorithm that places tightly connected pairs close together.

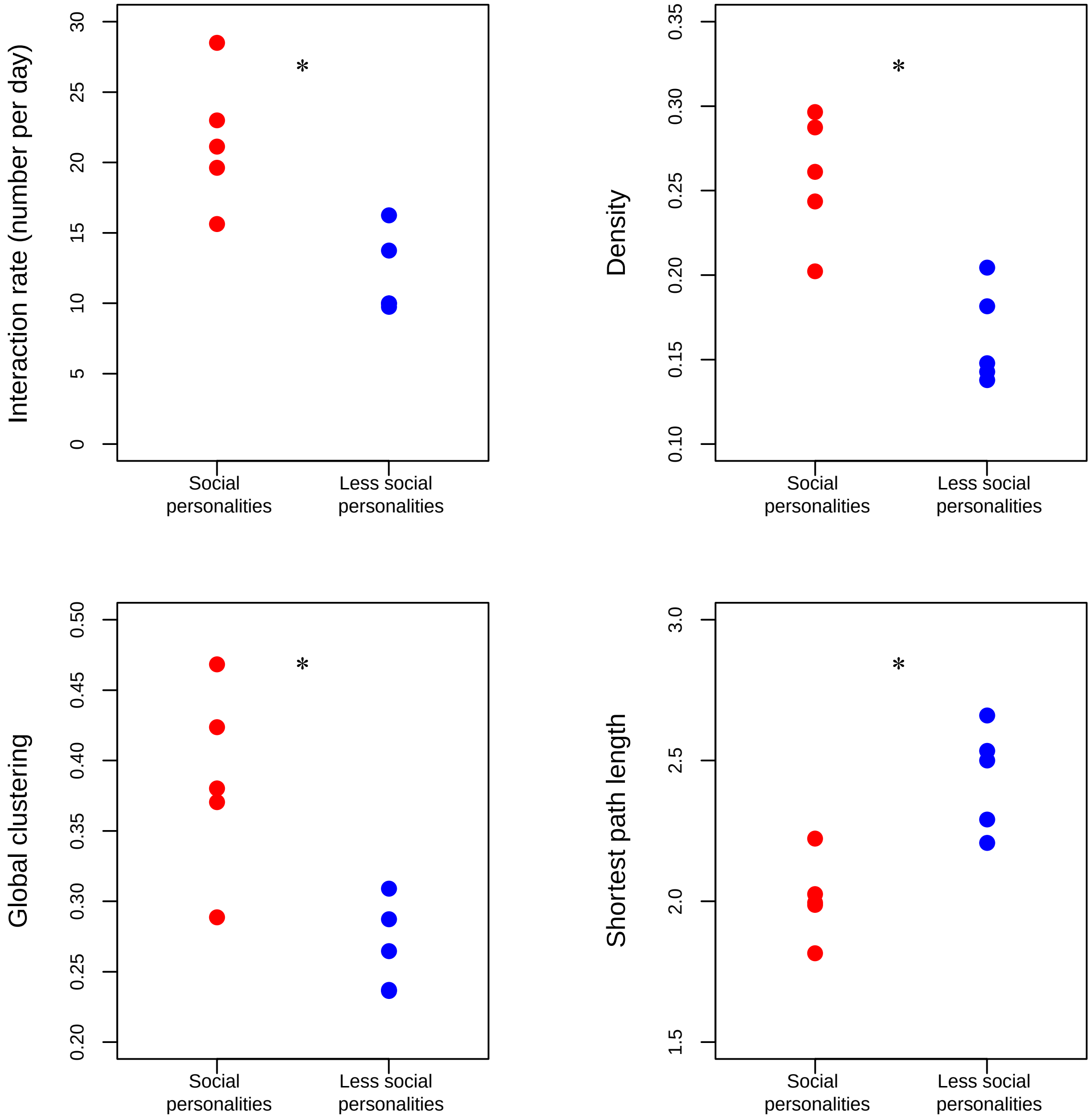

Figure 2. Four metrics of group network structure for the ten experimental populations. Each point represents one population. Statistically significant differences between the treatments are indicated by asterisks.

SUPPLEMENTAL MATERIALS

Constructing Replicate Networks

We constructed 10 screened enclosures of the same size (2.4x2.4x1.2m) and design in a small (~75 m^2) area of forest on the grounds of Mountain Lake Biological Station in Pembroke, Virginia, all within 15m of one another. This layout ensured that they experienced similar environmental conditions, other than minor variation in tree cover and shade level in this area. Raised wooden shelves (2.34m x 0.52m) were constructed in each enclosure to hold 54 uniform 13cm x 17.4cm polypropylene filter bags ($SacO_2$) filled with hardwood sawdust, which acted as both a surface for beetles to walk on and substrate for fungus. These shelves were built roughly 1.5-2m high for ease of visual observation. Each shelving unit held 18 brackets of *Ganoderma tsugae* cloned from the same strain (Sharondale Mushroom Farm) and of the same size and age. In all enclosures, fungal brackets were distributed in three clumps of six across the shelves. Enclosures were constructed in pairs with a shared screen wall between the backsides of two log shelves to save on materials costs.

Population assignments were performed using random draws from sex-specific body size quantiles to ensure sex ratios and body size distributions were consistent across experimental replicates. This process could be repeatedly simulated until any additional requirements were met. For the initial round of surveying, there were no additional requirements; for the second round, we chose the simulated set of assignments that maximized the difference in average number of interactions during the first round between the treatments while minimizing the number of beetles within each newly created population that had been in the same population during the first round (in the final set of assignments, no more than eight previously interacting individuals were placed together in any population). Each adjacent pair of enclosures received one population from each treatment to avoid creating a correlation between treatment status and any undetected microenvironmental variation across the area. Within the pairs, the enclosure that contained each treatment was randomized during the population assignment process to remove any spatial pattern which could be easily guessed or remembered by the observers collecting data. All observers were kept blind to the treatment status of populations during data collection.

Visual surveys of populations were conducted three times a day, from 0630 to 0930, 1430 to 1630, and 2130 to 0030. Each of the 10 populations was surveyed during each of these blocks. Throughout both the initial phenotyping and the experimental manipulation, the order in which populations were surveyed was rotated to prevent any differences among replicates arising due to consistent order effects or diel effects. Observers were also rotated across both survey periods and enclosures. During a survey, all surfaces in an enclosure were scanned in a pre-defined order using both flashlights and UV lights to maximize detection of beetles labeled with fluorescent tags. All behavioural data went through a rigorous error-checking process to prevent creation of false network ties.

SUPPLEMENTAL FIGURES

Figure S1. A) A pair of enclosures constructed back-to-back, with another visible in the background. B) The shelf within an enclosure containing 54 bags of sawdust, 18 of them inoculated with fungus and producing bracket fruiting bodies. C) Three labeled beetles, a courting male and female pair and another male, interact on the edge of a fungus bracket. Photo credits: A and C, Caitlin McIver; B, Robin Costello.

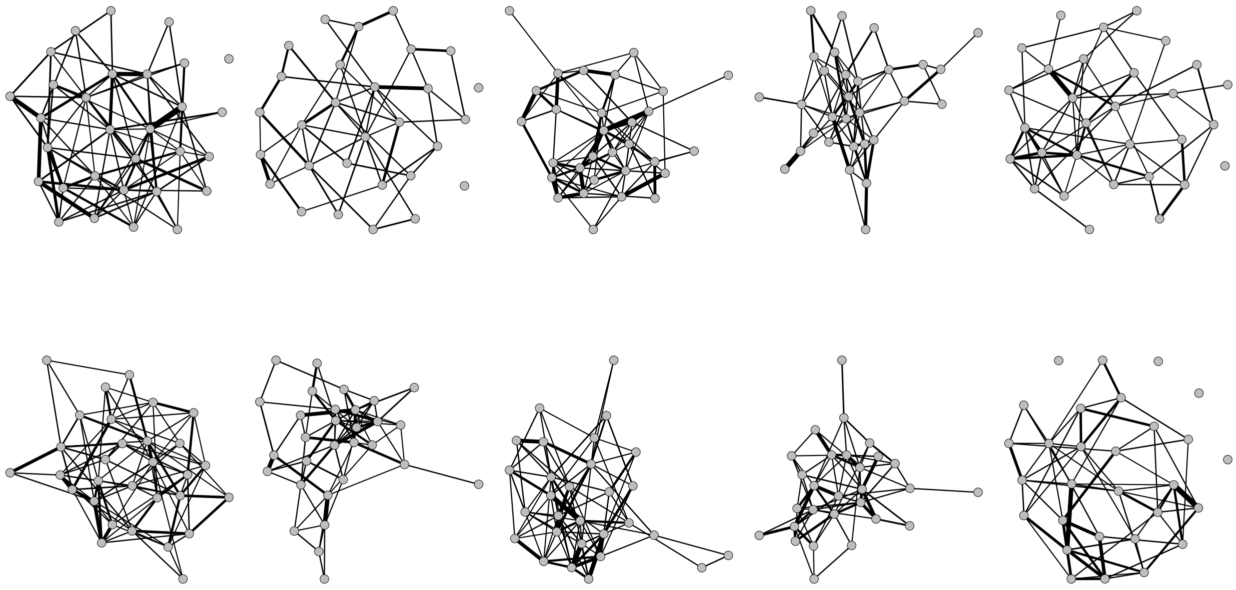

Figure S2. The initial networks used for phenotyping sociality. Line thickness is proportional to the simple ratio index, and nodes are plotted using an algorithm that places tightly connected pairs close together. Note that these networks are not directly comparable to the networks from the second half of the experiment, as they were sampled at different times.

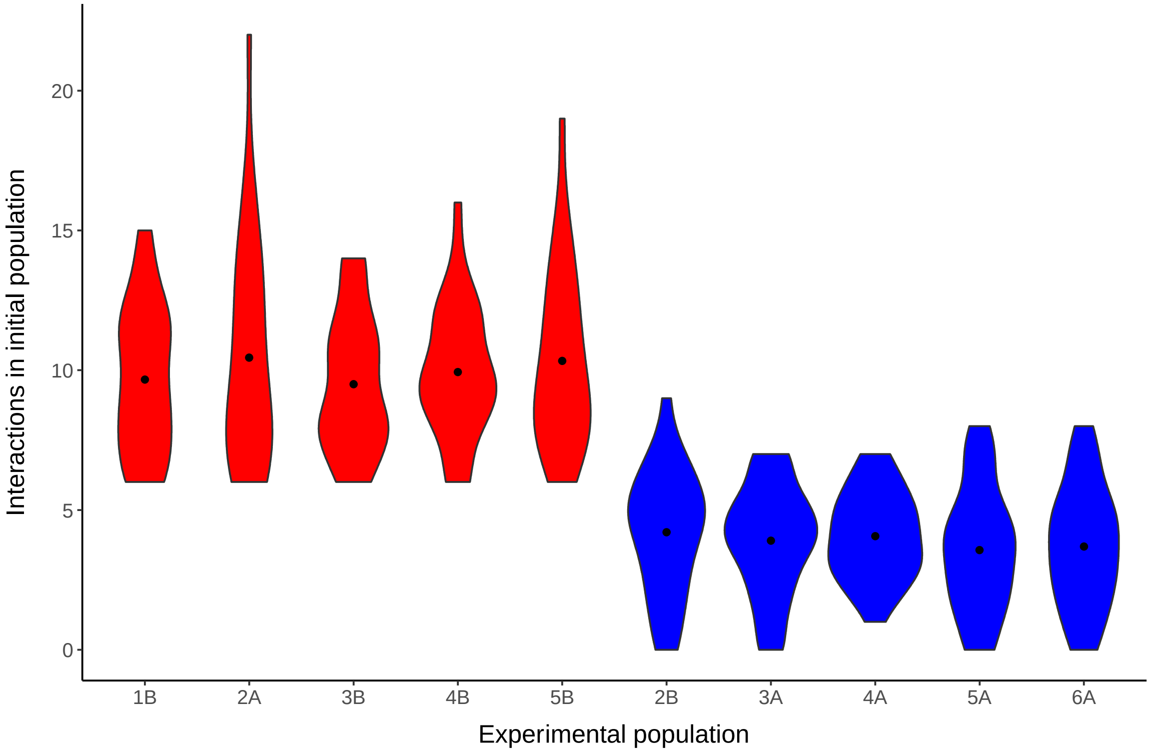

Figure S3. Distribution of initial measures of sociality in the experimental populations of the second round of the experiment, with means marked in black. The five “previously highly social” populations, in red, were composed of individuals who had more interactions in the initial populations (overall mean = 10, range = 6 – 22) than the five “previously less social” populations, in blue (mean = 4, range = 0 – 9). These ranges overlapped to satisfy the other assignment conditions and minimize demographic differences between the treatments.

CHAPTER TWO: Individual and population age impact social behavior and network structure in a long-lived insect[2]

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[2] Formatted as a coauthored manuscript: Cook, P. A., R. A. Costello, V. A. Formica, and E. D. Brodie III

Abstract

Variation in social behavior among individuals and groups has significant fitness consequences, and understanding the causes of such variation is important for being able to understand the progression of cultural changes or disease outbreaks. Here, we investigate the possibility that age contributes to variation in social behavior at multiple levels: within individuals over the course of their lives, among individuals of different ages within a group, among local social environments within groups, and among groups with differing age structures. We used experimental manipulations of captive populations combined with a multi-year longitudinal dataset to show that social behavior is associated with age across levels of organization in a long-lived insect, the forked fungus beetle *Bolitotherus cornutus.* In cross-sectional analyses of captive populations, older beetles were less socially connected and less central in their population social networks. A longitudinal study confirmed that this effect was due at least in part to changes in behavior over time; beetles phenotyped twice two years apart declined dramatically in number of social partners over that time, possibly because of increased social selectivity or a shift towards investment in reproduction towards the end of life. Beetles of different ages also occupied different social neighborhoods with a population. The effects of age on behavior scaled up such that populations of older individuals had fewer and stronger ties, longer path lengths, and lower clustering than populations of young individuals. Age therefore impacted not only individual exposure to information, disease, and other costs and benefits of sociality but also the network structures which mediate critical population processes.

Introduction

Patterns of social interactions among conspecifics shape a variety of ecological and evolutionary processes, from the transmission of information and pathogens (VanderWaal et al. 2014; Aplin et al. 2015; Stroeymeyt et al. 2018), to the expression, fitness consequences, and heritability of traits (reviewed in Fisher and McAdam 2017; Brodie et al. 2021). Understanding the causes of variation in social behavior is therefore of use in a wide range of biological and sociological disciplines. In recent decades, animal social network analysis has been used to describe patterns of social interaction at multiple scales of organization, from the immediate neighborhood an individual experiences to its position in the structure of its population and up to the emergent structure of the network as a whole. Each of these levels been demonstrated to have fitness consequences, through natural (Bond et al. 2021), sexual (Schülke et al. 2010), social (Brodie et al. 2021), or multilevel selection (Barocas et al. 2011b; Royle et al. 2012; Costello 2020). Although significant progress has been made towards understanding the sources of variation in individual social behavior, much less is known about when and why higher-order social structures vary in space and time. Here, we investigate the possibility that indivudal age may explain variation in social behavior at multiple scales of animal societies.

Age is associated with a suite of phenotypic changes, many of which could produce changes in social behavior. The net costs of mating and other interactions could all change with age or stage and potentially change social network position (Rodrigues 2018). Activity level and foraging patterns may also change, potentially resulting in shifts in social interactions. For example, red deer become less social with age, partially due to changes in spatial behavior (Albery et al. 2021). Other mechanisms include cognitive changes. Information accumulated over time can increase social competency and make older individuals sought-out social partners

(Jaatinen and Öst 2011; Taborsky and Oliveira 2012). Alternatively, cognitive senescence can decrease the ability to learn new information, potentially making older individuals less valuable partners and leading to their being avoided (Kulahci and Quinn 2019). Lastly, if reproduction or risk of mortality are age-dependent, individuals may alter their investments in social behavior as part of their life-history strategy (Tringali et al. 2020; Kroeger et al. 2021). All of these factors could create differences between old and young individuals in the social environment they experience and the positions they occupy within the overall population social network.

Individual change is not the only mechanism that can produce a relationship between age and social behavior. In humans and many other taxa, studies have found that sociality is correlated with lifespan, such that either more or less social individuals die earlier than others (reviewed in Korb and Heinze 2021). This selective disappearance means that the individuals surviving to old age represent a non-random sample of the initial population with respect to social behavior, creating a correlation between age and behavior. A similar result could be found if viability selection acts on a trait correlated with sociality, such as boldness or body size. In any of these cases, social behavior would not correlate with age within individuals, but will still correlate with age across individuals.

There is a rapidly growing body of research on sociality and age, but few studies have looked at levels of organization higher than the individual. At the level of the group, the composition of individual ages, or age structure, can also vary among populations or subpopulations. The field of population dynamics has done extensive work on the factors producing variation in age structures. Across a metapopulation, the age structure of subpopulations may differ between subpopulations due to differences in recruitment (Cooper and Shanks 2011) and local environmental factors such as predation regimes or harvesting (Miaud et al. 1993). Even within a population, age structure is not stable; fluctuations in age structure have been found to be of a similar magnitude to those in population size (Hoy et al. 2020). This suggests a possible source of variation in social networks: if individuals of different ages behave differently, we expect that the age structure of a population will shape its network structure, and therefore the many processes mediated by these structures. Social network structures are known to depend on the frequencies of individual physiological and behavioral traits (Cantor et al. 2021; Cook et al. 2022), but to our knowledge, no work has combined this with the growing body of knowledge about age-related behavioral changes.

We used an experimental approach to study the relationship between age and social interactions at several levels of social organization in a long-lived insect. Screened mesocosms in the natural habitat of our study system closely match natural conditions while removing extrinsic mortality from predation. These mesocosms also allow us to create replicate experimental networks, a powerful tool for understanding the drivers of variation in network structure (Krause et al. 2010; Smith et al. 2019). In this study we combine longitudinal data, phenotyping the social behavior of the same individuals in populations of the same size across multiple years, with an experimental manipulation of age structure that controls for other demographic variables. We manipulated the age structure of populations such that we could not only measure the relationship between individual age and social behavior, but also test whether the composition of a group with respect to age alters the structure of the group social network.

Materials and Methods

Study System

Bolitotherus cornutus, the forked fungus beetle, is a holometabolous tenebrionid beetle that lives on wood-decaying shelf fungi in the forests of eastern North America (Whitlock 1992). In the wild, they live clustered together into subpopulations on resource patches created by fallen logs. These fungi provide food, shelter, oviposition sites, and arenas for social interactions (Liles 1956; Pace 1967). Larvae, pupae, and newly eclosed adults develop inside the fungus fruiting bodies, or "brackets," for months to years before emerging as sexually mature adults. After emergence, many individuals only survive for one breeding season, but some have been recaptured for as long as five years (Formica, unpublished data). Insects make up over half of all described species (Mayhew 2007) and contain enormous inter- and intra- specific variation in both social behavior and lifespan. Despite this rich diversity, most studies of age in insects are limited to lab studies of *Drosophila* (Guo et al. 2020; Zajitschek et al. 2020; Quigley and Amdam 2021). Forked fungus beetles have been suggested as suitable for studies of aging and age-related variation in natural conditions (Zajitschek et al. 2020).

The age structure of forked fungus beetle subpopulations is highly variable. In the Pond Drain metapopulation near Mountain Lake Biological Station in southwestern Virginia, where beetles have been exhaustively collected and labeled since 2015, the proportion of beetles on a single log who are more than one year old ranges from 5% to 64% (Cook and Medina-Valencia, unpublished data). This variation may be caused by the age of the resource patch, local predation regimes, dispersal patterns, and other processes. Nothing is yet known about the consequences of age structure at the subpopulation level in this species.

At the individual level, variation in age is known to be associated with variation in dyadic social behavior. Older individuals of both sexes participate in more courting and mating interactions than do one-year-olds (Conner 1989; Phoebe A. Cook 2022 Chapter 2; Dos Anjos and Formica In prep). Older males initiate more aggressive interactions than one-year-olds (Mitchem et al. Submitted), which may make them more likely to win fights as aggression is associated with winning in male-male contests (Mitchem et al. 2019). It is unknown whether these differences between age are caused by longitudinal change, differential survival, or both.

For the past four years, we have maintained a captive breeding population of *B. cornutus* at Mountain Lake Biological Station (37°22'37.0"N, 80°31'17.5"W). This population was founded with wild beetles collected from the surrounding area in 2017 and 2018. Although the exact age of these founders at the time of capture is unknown, we know their minimum age. Several years of field surveys of a nearby metapopulation have found that 60% of all observations are of one-year-old individuals (unpublished data), so this minimum is likely correct for the majority of individuals and conservative for the rest. These individuals were allowed to breed in screen cages in the forest. Offspring develop in old fungal brackets, as in the wild. Each year we search the populations for newly emerged offspring and mark them with a unique three-character code affixed to their elytra with a UV-cured acrylic glue (Tuffleye Wet-A-Hook Technologies, San Antonio, TX). We therefore know the age of all individuals within the population in 2020: they could be tenerals (or “young of the year”) newly emerged late in the summer of 2020, two-year-olds first found in 2019, or wild-caught in 2017 and 2018 and therefore at least three years old.

Experimental Design

We tested the effects of both individual and social group age on behavior by creating two different experimental treatments. In the summer of 2020, we created twelve experimental populations of thirty-six beetles each, six replicates in both of two treatments. The six "young" populations consisted of eighteen tenerals and eighteen two-year-olds, and the six "old" populations consisted of eighteen two-year-olds and eighteen beetles that were three or more years old.

We minimized differences among these populations in sex ratio, body size, relatedness, and past interaction history. The body size of each individual was measured as the length of the elytra from an image taken on a flatbed scanner (Epson Perfection V600 Photo) using ImageJ (Abramoff et al. 2003). We then used structured sampling from sex- and age- specific size quantiles to create populations that had equal sex ratios and did not differ from each other in body size ($F_{11,425} = 0.22$, $p = 0.996$) (see Cook et al. 2022 Supplemental Material for details). This process was repeatedly simulated until the population assignments also minimized the number of beetles placed together who had overwintered or emerged from the same enclosure. All were held in isolation for at least seven days before the start of the experiment, which past studies suggest is enough time for patterns of social interaction to "reset" (Formica et al. 2017).

Populations interacted freely within 2.4 by 2.4 by 1.2 m screened experimental enclosures built to mimic natural resource patches in the forest. Enclosures contained mulch floors and "artificial logs," wooden shelving units holding 54 bags of hardwood sawdust. Eighteen bags were inoculated with the same strain of the *B. cornutus* host fungus species *Ganoderma tsugae* (Sharondale Mushroom Farm) and allowed to produce brackets. These shelves mimic the logs on which beetles live in the wild, but with fungus age, size, genotype, and spacing all controlled so as not to vary between populations. The screened enclosures are placed in an area of forest where this species naturally occurs and are exposed to natural abiotic conditions. At the start of the experiment, individuals were allowed to acclimatize to the enclosures for 36 hours before behavioral observation began. After acclimatization, we performed scan sampling of dyadic interactions three times a day (0630-0930, 1430-1630, and 2130-0030) for twenty-one days in August of 2020. This time of year is within the breeding season, and by this point many of the young of the year will have eclosed and emerged. Individuals were defined as social partners if they were in physical contact or close proximity (within 5 cm) of one another, excluding mating interactions, which are not included in measures of sociability (Gartland et al. 2021)

Observers could not be made blind to the treatment because the individual identification codes used to label beetles have progressed in a predictable sequence over the years. However, the majority of the observers were not aware of the questions that would be answered with the data, and a priori predictions as to the direction of effects had been made when data collection was underway. Both the order in which the populations were surveyed and individual observer identity were randomized to control for possible time and observer biases.

Network Creation

We constructed a social network from the non-mating interactions, as defined above, between all individuals in each of the twelve populations. Ties in these networks are undirected and weighted by the simple ratio index, which describes associations as the fraction of times that a pair was observed together out of the total observation periods at least one was observed (Ginsberg and Young 1992). Our 63 observation periods are sufficient to estimate association strengths precisely (Whitehead 2008).

Twenty-three beetles died during the experiment: 3 of 108 tenerals, 6 of 219 two-year-olds, and 14 of 110 that were three years or older. Those that died in the first three days of surveying were replaced with beetles of the same age, sex, and size. Individuals who died were included in the networks, for accurate description of social structures and environments, but removed from other analyses. Any observations that could not be confidently assigned to an existing beetle ID were excluded from network creation to avoid the creation of false nodes. Our final dataset consists of twelve networks comprised of 4,409 interactions between 414 individuals. Networks were visualized using the R package igraph (Csardi and Nepusz 2006). All analysis was performed in R version 4.1 (R Core Team 2021).

Individual Age and Network Position

We compared three measures of social network position between beetles of different ages. *Strength* is a measure of local connectivity, quantifying the number and weight of all a node's ties. We calculated strength using a tuning parameter of 0.5, meaning that each additional partner increases strength by 1 and repeated interactions increase strength by 0.5 each (Opsahl 2009). *Betweenness* measures an individual's centrality in the network as a whole. Individuals along paths connecting many dyads in the population have high betweenness and potentially mediate the flow of information or disease. *Clustering coefficient* is a measure of cliquishness, calculated as the proportion of an individual's social partners who interact with each other (Croft et al. 2011). An individual who primarily interacts with an existing social group would have high clustering, while a broadly interacting individual would have lower clustering. These metrics have previously been shown to be under selection in this species (Formica et al. 2012, 2020), and strength and betweenness are known to be repeatable on the scale of weeks (Formica et al. 2017).

We modeled each of these three metrics of network position using generalized linear mixed models. Body size and number of times seen have previously been shown to explain variation in social behavior in this system, so these covariates were included along with age. Both were globally standardized to improve model fits. A sex-by-age interaction allowed us to test whether the relationship between age and sociality differs between males and females. We did not include other interactions terms because we had no a priori reasons to do so. A fixed effect of treatment (young or old population) and a random effect of population were also included, to account for differences among populations. The strength and clustering models were fit with a tweedie error distribution and the betweenness model with a negative binomial distribution in the R package glmmTMB (Brooks et al., 2017), with assuming zero-inflation was constant across the dataset. Assumptions of residual homogeneity, dispersion, and zero inflation were tested visually and statistically with DHARMa (Hartig 2021).

To test the statistical significance of the effects from these models, we compared the F-statistics from each model run in our observed data to a distribution of F-statistics from the same model run in 1000 permuted datasets. Permutation tests are required for inferences about individual network data because the network positions of members of the same population are non-independent of one another, making conventional statistical tests inappropriate (Croft et al., 2011; Farine & Whitehead, 2015; Farine, 2017). Following Costello et al. 2020, we used a node permutation approach suitable for our experimental design. Node permutations are less prone to false positive errors than other permutation approaches (Croft et al., 2011; Puga-Gonzalez et al., 2020; Weiss et al., 2021). Each permutation randomly shuffled all variables without replacement among all individuals to break any covariances with network position or with the other covariates. We ran the analyses described above in each permuted dataset, creating a set of 1000

F-statistics for each variable in each of the three models. P-values were calculated as the proportion of the permuted estimates more extreme than the observed estimate.

After the above analyses were completed, a post-hoc analysis used an additional dataset to investigate whether differences in social network position between age classes are due to changes over an individual's lifetime, rather than an association between social behavior and early mortality. An experiment in the summer of 2018 created populations of the same size and sex ratio that were also sampled with the methods described above (Costello 2020). The oldest beetles in our experiment were phenotyped in this 2018 study, and we took advantage of this to ask whether their social behavior changed over two years. We calculated the change in number of social partners, or network degree, from 2018 to 2020 for the 96 individuals in both experiments. To account for weather and other factors that might influence activity level, we compared this to the differences in degree between 96 randomly selected young beetles in 2018 and another 96 young beetles in 2020 using a two-sided t-test.

Individual Age and Age of Social Partners

We asked whether individuals assort by age, such that individual's age is associated with the age of their immediate social environment. Mean social partner age, weighted by frequency of interaction, was calculated for each individual and compared between age classes using t-tests. To prevent the result from being forced positive as an artefact of the experimental design, in which old and young individuals were sorted into different populations, these tests were run separately in the two treatments.

Population Age and Network Structures

We tested whether population age structure affects five metrics of global network structure. The *total number of interactions* is simply how many non-mating social interactions occurred. *Tie density* quantifies how many of all possible dyads interacted, and the *coefficient of variation* of tie weight measures how unevenly interactions were distributed among those pairs. *Global clustering coefficient* is determined by how many possible triads are closed. The *average shortest path length* measures how closely linked the network is. Tie densities were calculated in the package igraph (Csardi and Nepusz 2006) and weighted clustering and shortest path lengths were calculated in tnet (Opsahl 2009).

Unlike individual-level metrics, measures of network structure are independent of one another and can be compared with parametric statistical tests rather than permutations (Croft et al. 2011). The comparison for groups of equal size sampled with equal effort under the same conditions is simple, as the null hypothesis is that network structures do not differ due to “nuisance effects” (James et al. 2009; Farine and Carter 2021). We used two-sided t-tests to compare each of the five network metrics between the age treatments, with six replicates in each treatment. A similar experiment with only ten populations showed significant effects of manipulating group composition—in that case, with regards to individual personality rather than age—on four of these metrics (Cook et al. 2022), suggesting that this design and sample size is powerful enough to detect strong differences in network structures between treatments.

Results

Individual Network Position

The oldest beetles in our experimental populations were less connected (individual age is associated with lower strength, Table 1) and less central (lower betweenness, Table 2) than the teneral and two-year olds, but did not differ from them in cliquishness (no difference in clustering coefficient, Table 3). There was no significant interaction between age and sex in any model, meaning that these patterns did not differ between males and females (Figure 1). Individuals in the older populations had lower betweenness (Treatment effect, Table 2), and there was a non-significant trend towards lower individual strength in these populations as well (Treatment effect, Table 1). Number of scans active was positively correlated with both strength and betweenness (Tables 1 and 2), but not clustering coefficient (Table 3).

In the longitudinal data, the 96 beetles phenotyped in both 2018 and 2020 declined in network degree over that time from an average of 12.4 social partners during a three-week study period in 2018 to 7.0 partners two years later (Figure 2). This decline was significantly greater than the difference in degree between the "control" groups of randomly selected young beetles in each year ($t = -4.32$, $df = 185.82$, $p < 0.001$).

Individual Age and Age of Social Partners

In both treatments, individual age was positively associated with the mean age of the social partners with whom they interacted. In populations composed of younger beetles, tenerals had lower average social partner ages than two-year-olds ($t = -6.97$, $df = 199.08$, $p < 0.0001$). In populations composed of older beetles, two-year-olds had lower social partner ages than beetles three years old or older ($t = -3.69$, $df = 146.65$, $p = 0.0003$).

Network Structure

Populations in the two age composition treatments had the same total number of non-mating interactions ($t = -1.17$, $df = 7.10$, $p = 0.280$), but the distribution of these interactions across the network differed with age structure (Figures 3 and 4). Populations composed of older individuals had lower tie densities, ($t = -3.92$, $df = 8.65$, $p = 0.003$), higher variation in tie weight ($t = 3.18$, $df = 8.28$, $p = 0.012$), lower global clustering ($t = -2.27$, $df = 9.14$, $p = 0.049$), and longer average shortest path lengths ($t = 4.98$, $df = 9.26$), $p < 0.001$) than populations with younger average ages.

Discussion

We find that age explains variation in social behavior at multiple scales. At the individual level, we find a correlation between age and social network position, caused at least in part by within-individual change in sociality over time. Additionally, we find positive assortment between individual age and the age of social partners, meaning that beetles of different ages are experiencing different local social environments even within the same population. At the group level, population age composition determines overall social network structure. This is, to our knowledge, the first demonstration that age structure shapes emergent social structures, and potentially processes such as social selection and disease transmission. The combination of our longitudinal data, experimental manipulation, and analysis at multiple scales allows us to understand both the causes and consequences of age-related variation among individuals.

Older beetles of both sexes are less connected and occupy more peripheral social positions in their populations than do young beetles. In the cross-sectional analysis, we see that beetles in the oldest age class have lower strength and lower betweenness than the other age classes. Looking at these same beetles over time, we see that they have declined dramatically in their number of unique social partners, much more so than can be accounted for by differences in baseline activity rates between the two years. We therefore conclude that individuals are changing longitudinally. This does not rule out the possibility that selective disappearance occurs as natural selection acts on social traits, either reinforcing the within-individual change (if highly social individuals are more likely to die early) or opposing it.

Longitudinal changes in social behavior might be due to accumulated social experience or intrinsic change. A plausible source of intrinsic change often invoked in life history evolution is the increased risk of mortality with age. Although this experiment was not designed to measure survivorship curves, mortality during the experiment was higher in our oldest age class than in the younger two classes. Further work could investigate whether this effect holds; if older individuals do indeed have higher mortality, they might be altering their behaviour as part of a terminal investment strategy. Regardless of the cause, reduced social connectivity likely means that older beetles have lower exposure to parasites, pathogens, and social information than young ones. More generally, our results suggest that age is an axis of variation we must account for in studies of social behavior.

Individual age is also weakly but positively correlated with the age of social partners, suggesting positive assortment by age even among the artificially constrained social options offered in the experimental enclosures. This assortment may reinforce effects of age on social behavior, because the most and least social age groups are tending to interact only with similarly behaving partners. Age homophily might be due to passive processes, such as older beetles sharing similar resource use and activity patterns due to age-related changes, active processes such as assortative mate preferences, or younger individuals avoiding competitive interactions with aggressive older beetles, or cohort effects (Brodie et al. 2021). Whatever the mechanism, the outcome is that individuals of different ages experience different social environments, even within the same population. The fitness consequences of social interaction may depend on the age of social partners (Rodrigues 2018), as the pattern of assortment will determine the effects of social selection (Brodie et al. 2021).

The individual-level effects of age scale up, such that the age structure of a population shapes its global social network structure. Populations of older individuals have fewer and more variable ties, longer path lengths, and lower clustering than populations of young individuals. This is, to our knowledge, the first demonstration that age structure can shape emergent social structures. Social network structure is hypothesized to change many population processes including disease transmission, and a few studies have shown evidence of multilevel selection on network structure (Royle et al. 2012; Costello et al. 2020). Further work should investigate whether population age structure alters group member fitness via these effects on network structure. If so, this would be a novel path by which age structure can affect population dynamics.

Group age composition can also influence individual social network position, sometimes in counter-intuitive ways. There was a non-significant trend in our results that individuals in the younger populations had higher strength, which is consistent with the finding from the longitudinal analysis that beetles decrease in strength over time. However, individuals in younger populations had lower centrality, as measured by betweenness, despite the fact that young individuals tended to be *more* central to their networks. This is not the paradox it may appear. Populations with younger age structures tend to be quite well and evenly connected, as seen in their high tie density and global clustering, and therefore connections are not tightly funneled through a few highly central nodes. This suggests that the most central individuals are the relatively young members of old populations, which potentially exert high influence on flows of parasites, pathogens, or information through the network.

Careful comparisons across taxa can identify both common patterns and diversity in age-related social changes. A decline in social connectivity with age has been interpreted as “social senescence” in red deer (Albery et al. 2021), but there are several reasons not to assume that a reduction in non-mating interactions constitutes deterioration in all cases. Both competitive ability and mating success increase from the first to third year of adulthood in forked fungus beetles (Mitchem et al. Submitted; Dos Anjos and Formica In prep). Instead of senescing, beetles may be shifting their energy to invest in reproductive interactions instead of non-mating interactions which expose them to costs of social interactions, such as disease, without major fitness benefits. Similarly, our results suggest beetles are shifting from interacting indiscriminately early in life to having fewer but less randomly distributed social ties as they age. This fits the pattern of increasing “social selectivity” with age, a phenomenon well documented in humans and other primates (Almeling et al. 2016; Rosati et al. 2020), and potentially of benefit for any long-lived species with a slow pace of life (Silk and Hodgson 2021; but see Bond et al. 2021). Using different frameworks and methods but possibly identifying related trends, other recent studies have found that older female eiders can afford to form smaller brood-rearing coalitions (Jaatinen and Öst 2011), older elephants have more stable network positions (Murphy et al. 2019), garter snakes become more selective in their associations over time (Skinner and Miller 2020), and older Arabian babblers occupy more similar positions across multilayer interactions than do young individuals (Dragić et al. 2021). A larger body of data on sociality and ageing in diverse long-lived organisms will help shed light on whether these are truly comparable trends and perhaps even represent similar mechanisms operating across species.

Acknowledgments and Funding

We thank Savanna Cabrera, Hannah Donald, and Sarah McPeek for help with data collection; Savanna Cabrera and Ahema Gaisie for data entry; and Alejandro Medina-Valencia for data cleanup. The staff of Mountain Lake Biological Station provided advice, support, and resources. Funding for this work was provided by an NSF Graduate Research Fellowship for P.A.C., a University of Virginia Double Hoo research award to P.A.C., NSF IOS-1355029 to V.A.F. and IOS-1355003 and DEB-1911485 to E.D.B.III, and NSF REU grant 1461169 to Mountain Lake Biological Station.

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Tables and Figures

Table 1. Estimates for fixed effects from the glmm explaining variation in strength. Estimates (slopes) are reported for continuous variables, and back-transformed marginal means for categorical variables. P-values are calculated as the proportion of F-statistics from the models run in permuted datasets which are greater than the observed model F-statistic. P-values significant at the $\alpha = 0.05$ level are indicated in bold.

Predictor of strength	Estimate	P-value
Body size (mm)	-0.02	0.557
Scans active	0.13	**0.001**
Individual age	one: 1.67 two: 1.69 three+: 1.17	**0.001**
Sex	F: 1.56 M: 1.42	0.089
Individual age x sex	-	0.904
Age composition	Young: 1.61 Old: 1.38	0.064

Table 2. Estimates for fixed effects from the glmm explaining variation in betweenness. Estimates are reported for continuous variables, and back-transformed marginal means for categorical variables. P-values are calculated as the proportion of F-statistics from the models run in permuted datasets which are greater than the observed model F-statistic. P-values significant at the $\alpha = 0.05$ level are indicated in bold.

Predictor of betweenness	Estimate	P-value
Body size (mm)	0.01	0.778
Scans active	0.25	**0.001**
Individual age	one: 24.8 two: 26.8 three+: 15.4	**0.007**
Sex	F: 24.4 M: 19.3	0.057
Individual age x sex	-	0.626
Age composition	Young: 18.7 Older: 25.2	**0.032**

Table 3. Estimates for fixed effects from the glmm explaining variation in clustering coefficient. Estimates are reported for continuous variables, and back-transformed marginal means for categorical variables and interactions. P-values are calculated as the proportion of F-statistics from the models run in permuted datasets which are greater than the observed model F-statistic. P-values significant at the α = 0.05 level are indicated in bold.

Predictor of clustering	**Estimate**	**P-value**
Body size (mm)	0.01	0.496
Scans active	-0.03	0.144
Individual age	one: 0.413 two: 0.422 three+: 0.404	0.669
Sex	F: 0.409 M: 0.417	0.646
Individual age x sex	-	0.766
Age composition	Older: 0.395 Young 0.431	0.168

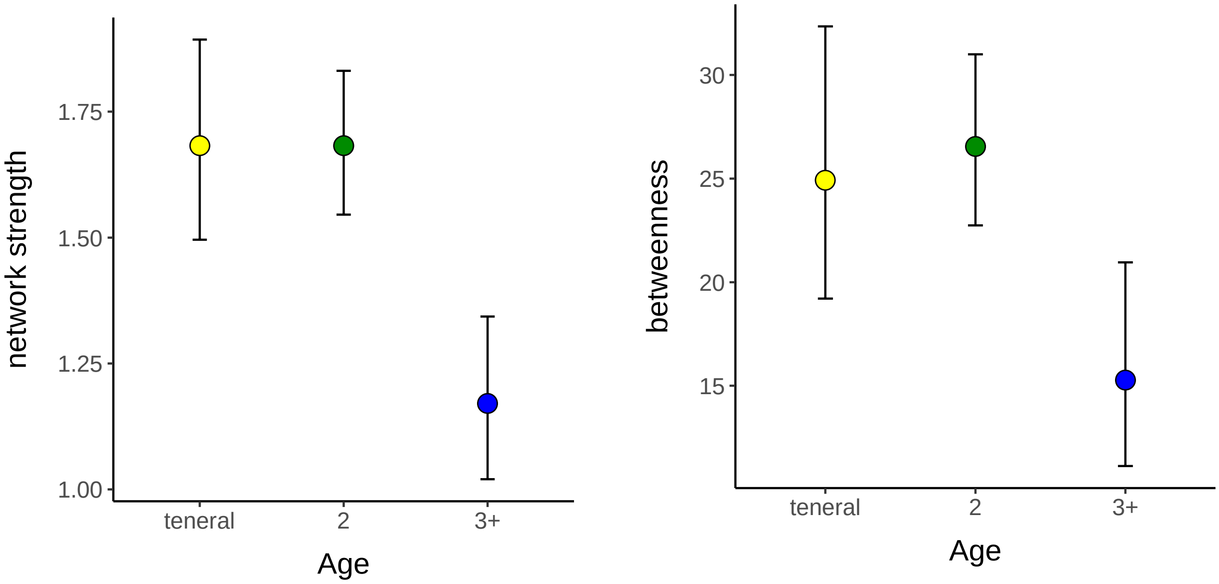

Figure 1. Back-transformed marginal means of strength (left) and betweenness (right) for the three age cohorts. The sexes have been combined here because the effects do not differ by sex. Error bars represent 95% confidence intervals.

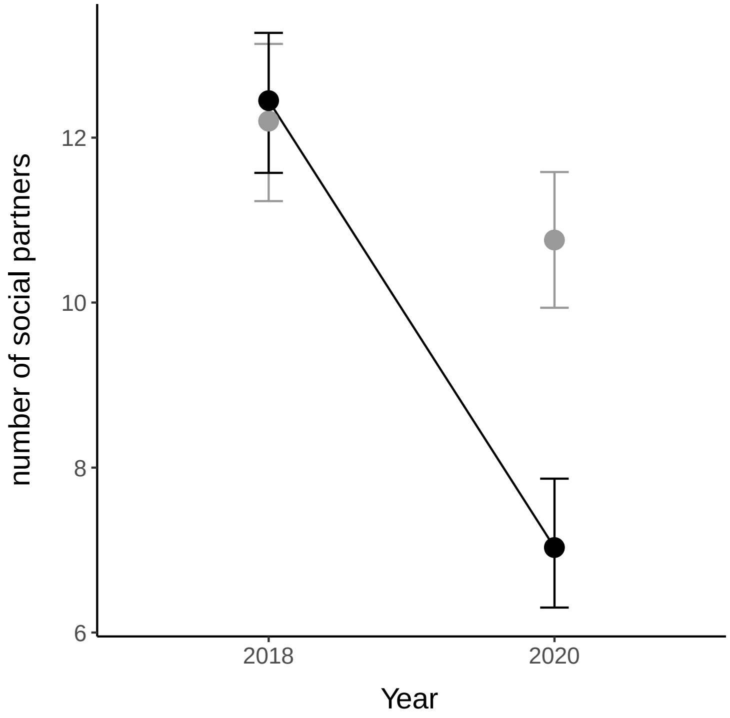

Figure 2. Change in number of partners (network degree) over time for the same individuals phenotyped in both 2018 and 2020 (black, connected by black line), compared with two groups of randomly selected young beetles in each year (grey). Error bars represent bootstrapped 95% confidence intervals around means.

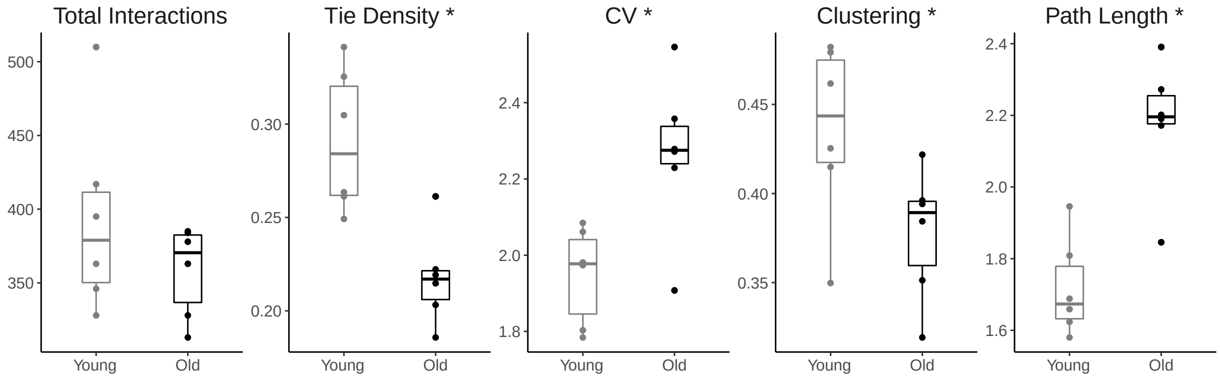

Figure 3. Five metrics of group network structure for the twelve experimental populations, by age composition. Each point represents a network of 36 individuals. Asterisks denote metrics which differ significantly between the young and old populations.

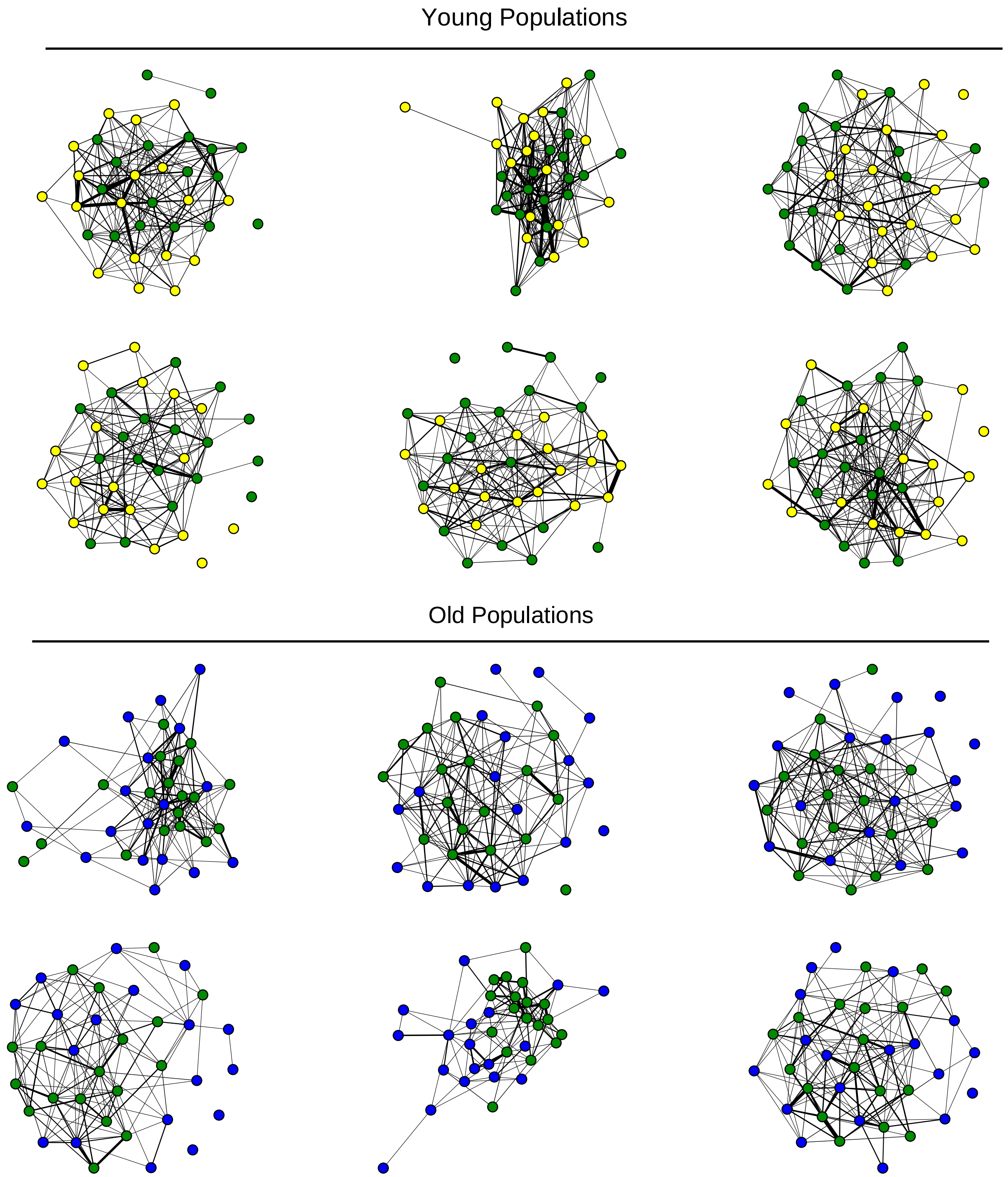

Figure 4. Networks from six populations composed of tenerals and two-year-olds (top two rows) and six composed of two-year-olds and three-year-olds (lower two rows). Lighter nodes represent younger beetles, and darker nodes older beetles. Tie thickness is proportional to the simple ratio index, and nodes are plotted using an algorithm that places tightly connected pairs close together. Three-year-olds (blue nodes) have fewer connections and are less central to their networks than members of the other age classes. Old populations have sparser and more variable ties, fewer closed triads, and longer paths between individuals than young populations.

CHAPTER THREE: Population age structure shapes individual fitness and phenotypic selection in a long-lived beetle[3]

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[3] Formatted as a coauthored manuscript: Cook, P. A., R. A. Costello, V. A. Formica, and E. D. Brodie III

Abstract

Although demographic factors such as operational sex ratio explain some variation in sexual selection across space and time, we know much less about other ecological drivers of sexual selection. We hypothesized that the fitness consequences of interactions will depend on the age of social partners, and therefore that population age structure may shape evolutionary pressures on sociality. Here, we investigate the consequences of age variation at multiple levels of social organization for both individual fitness (mating success of males and number of eggs laid by females) and sexual selection on a social network trait. We experimentally manipulated the age composition of populations of the forked fungus beetles *Bolitotherus cornutus,* creating twelve replicate mesocosm populations with either young or old age structures. We found that older beetles have higher reproductive success than newly emerged tenerals, and the age of the social environment also matters: males pay a fitness cost when their social interactions are primarily with old males, and both sexes achieve lower fitness in old populations. In addition to explaining variation in individual fitness, population age structure also altered the sexual selection acting on female social network position. Female sociality does not experience selection in young populations, but is under positive directional selection in old populations. Our results highlight age structure as understudied demographic factor which may shape the landscape of sexual selection, contributing to our understanding of when and why we observe variation in selection regimes.

Introduction

Sexual selection regimes are shaped by demographic factors. The relationship between sexual selection and factors such as operational sex ratio (Emlen and Oring 1977; Kvarnemo and Ahnesjo 1996); population density, size, and crowding (Eshel 1979; Conner 1989a; Shuster and Wade 2003); and group phenotypic composition (Sih and Watters 2005; Eldakar et al. 2009) has been well studied. It is surprising, then, that population age structure has been largely neglected in this area. The composition of individual ages within a population or deme can depend on time since founding, connectivity and recruitment, density, climate, severe weather events, intraspecific competition, and parasitism or predation (Levins 1969; Miaud et al. 1993; Coulson et al. 2001; Festa-Bianchet et al. 2003; Wright et al. 2006; Hoy et al. 2015). Age structures of populations or subpopulations are thus highly variable in space and time (Hoy et al. 2020). The consequences of this variation for population dynamics have been extensively studied by ecologists. Less attention has been given to possible consequences for the strength and direction of sexual selection, as age structures alter the social environments experienced by group members.

Individual age is associated with variation in information (McComb et al. 2001; Jaatinen and Öst 2011), foraging ability and energy reserves (Hendry and Berg 2011; Patterson et al. 2016), and immune function and infection status (Reavey et al. 2015; Leech et al. 2019), as well as a variety of social behaviors (Almeling et al. 2016; Rosati et al. 2020; Albery et al. 2021b), competitive ability (Jones et al. 2007; Baxter and Dukas 2017), and mating success or extra-pair paternity (Conner 1989b; Forslund and Pärt 1995; Isaac and Johnson 2005, Roth et al. 2019). Many of these traits will impact exposure to risks and access to resources and mates for other members of the population. Every individual is part of the social context for conspecifics, whether through direct social interactions or though shared space and resources. When looked at from a different direction, an individual’s fitness may depend on the age of the conspecifics with whom that individual either directly interacts or shares group membership.

If the fitness consequences of social interactions depend on the age of social partners, then we would expect selection on social traits to depend on the ages of all potential partners (Rodrigues 2018). Population differences in selection on social behavior would therefore vary with the age structure of populations—being highly social will be beneficial when interactions with partners increase fitness, but not when interacting is costly. Due to their labile and interactive nature, social traits are expected to experience more context-dependent selection than other traits (Eldakar et al. 2010; Bailey et al. 2018; Formica et al. 2020; Turner et al. 2020).

Social network position is one way of describing variation in social behavior. Metrics of social network position describe where an individual falls within the social structure of all interactions within a population, which modulates exposure to information, parasites, disease, grooming, and other positive and negative fitness consequences of social interactions (Flack et al. 2006; Drewe 2010; Formica et al. 2012; Claidière et al. 2013; Dey et al. 2013; VanderWaal et al. 2014b; Aplin et al. 2015a; Carter et al. 2020; Gartland et al. 2021). Many of these fitness consequences of interaction are due to traits associated with age, and so the net fitness effect of sociality will depend on the age of possible partners. We hypothesized that selection on an individual’s position in the social network of a population will depend on the age structure of that population.

We studied how the age of the social environment, both that of directly interacting partners and all members of the population, shapes fitness and selective landscapes in the forked fungus beetle *Bolitotherus cornutus.* Selective pressures are well studied in this species. Early work established that male horn and body size are under positive natural and sexual selection; larger males live longer and have higher reproductive success (Conner 1988, 1989a). In a classic example of demography shaping sexual selection, selection on horn length is stronger in subpopulations where the density of males relative to fungal brackets in the population is low (Conner 1989a). Male morphology is also under opposing social selection. Males who interact with large male social partners pay a fitness cost (Formica et al. 2011). Social behavior is under more variable selection. Individual social network position is correlated with reproductive success in this species, but much less predictably so than morphology, and the mechanisms of this selection are unknown. The strength and even direction of sexual selection on network position are highly variable among wild subpopulations (Formica et al. 2020). Work in replicate mesocosm populations has shown that selection on social network position differs between populations with different spatial distribution of resources (Costello et al. In press). Wild subpopulations vary in many other physical and demographic variables, including age structure (Whitlock 1992, Medina-Valencia unpublished data).

Forked fungus beetles are long-lived. Although roughly a third of adults survive for less than a month after emergence (Conner 1989b), some have been recaptured as long as five years after initial capture (Formica personal communication), leading to significant variation in age within a population. Age is associated with variation in several behavioral traits. Older males are more aggressive (Mitchem et al. Submitted), and may therefore be more likely to win fights, as aggression is associated with contest outcomes in male-male interactions (Mitchem et al. 2019). Individuals of both sexes alter their social behavior as they age, participating in fewer non-mating social interactions and occupying more peripheral positions within the social network of all interactions within their population (Cook Chapter 2). These changes scale up, such that the overall social network structure of a subpopulation depends on its age composition (Cook Chapter 2). Older individuals also invest more in reproductive interactions, gaining higher reproductive success (Conner 1989b; Dos Anjos and Formica In prep).

Given the extensive age-related variation and unexplained variation in selective pressures on social behavior documented in this system, we hypothesized that population age structure impacts both individual fitness and sexual selection through changes in the behavioral environments experienced by individuals. We experimentally manipulated mesocosm populations to ask whether individual age, social neighborhood age, and population age explain variation in individual reproductive success, and whether age structure shapes selection on sociality. This approach allows for strong experimental tests of how population demographic factors alter selection regimes, and has been effective in past work (Costello 2020). Controlled replicate populations are especially useful for answering otherwise intractable questions about social behaviors (Krause et al. 2010; Smith et al. 2019).

Materials and Methods

Study System

Bolitotherus cornutus is a long-lived tenebrionid beetle found throughout the forests of eastern North America (Whitlock 1992). The metapopulation occupying a region of forest is spatially subdivided into subpopulations, each consisting of the beetles living on single fallen log. These logs provide resource patches of the wood-decaying fungi in which larvae and pupae develop and on which adults feed, mate, and oviposit (Liles 1956; Pace 1967b). Males of *B. cornutus* use their thoracic horns in competitive interactions over access to females. Males vary significantly in both body and horn size. Larger horns allow a male to pry mate-guarding competitors off of females, and larger body size can make a male difficult to remove. This species is holometabolous—after they eclose and emerge from the fungus brackets, adults do not molt or grow over time. Size is therefore not associated with age.

Since 2017, we have maintained a captive breeding colony of *B. cornutus* at Mountain Lake Biological Station (37°22'37.0"N, 80°31'17.5"W). Colony founders were collected from the surrounding area in the spring of 2017 and 2018. These founders have been allowed to interact and reproduce within 2.4 by 2.4 by 1.2 m screened experimental enclosures built to mimic natural resource patches in the forest where this species naturally occurs. Each enclosure contained 18 brackets of the *B. cornutus* host fungus species *Ganoderma tsugae* (Sharondale Mushroom Farm) growing out of bags of hardwood sawdust held on wooden shelves. These shelves act as “artificial logs.” Individuals in the colony have thus been allowed to experience semi-natural conditions but shielded from predation and prevented from emigrating. Each year at the start of the season we collect the offspring which emerged in late fall or early spring, and we continue to search for new offspring through the field season until the end of the summer. Newly caught offspring, who may emerge any time from early spring to late fall, are marked with a unique three-character code affixed to their elytra, and each individual is imaged with a flatbed scanner so that even if both labels are lost they can still be identified. The founders, adult in spring of 2017 or 2018, were therefore at least three years old during our experiment in the summer of 2020. Offspring collected in early 2019 are considered two-year-olds in 2020, as they likely emerged in fall 2018, and tenerals collected over early summer 2020 are one-year-olds or “young of the year.”

Experimental Design and Data

In early August of 2020, 432 beetles from the age three cohorts were placed into twelve experimental populations, six in each of two different treatments. Six “young populations” consisted of eighteen tenerals and eighteen two-year-olds, and six “old populations” consisted of eighteen two-year-olds and eighteen beetles that were three or more years old. Both the physical environment (enclosure size and layout; fungal resource age, size, genotype, and distribution in space) and population demographics (population size and density, sex ratio, and average body size) of the replicates were controlled, with the only difference between treatments being age structure. The density of males per fungus bracket in each population was comparatively low, creating conditions expected to lead to strong sexual selection (Conner 1989a). Individuals were kept in isolation for a minimum of seven days before being placed into the enclosure to allow patterns of social interaction to “reset” (Formica et al. 2017a) and allowed to acclimatize to the enclosures for 36 hours before we began behavioral observations.

We performed scan sampling of behavior three times a day (0630-0930, 1430-1630, and 2130-0030) for twenty-one days in August of 2020, noting the behavior and social partners of all visible, identifiable individuals. Male reproductive success was estimated as the number of successful insemination events, which can be identified from the stereotyped mate guarding behavior that follows (Conner 1988). Female reproductive success was estimated as the number of observed oviposition events, which is the same as the number of eggs laid because females lay only one egg at a time. Observer identity and the order in which populations were surveyed were randomized every survey period, to prevent treatment from being confounded with possible effects of time or observer identity.

To characterize each individual’s local social environment, we recorded all social interactions. Social partners were defined as individuals in close proximity (physically touching or within 5 cm of each other), but excluded courting, copulating, and mate-guarding males and female pairs to allow for the measurement of selection without creating autocorrelation between our social phenotypes and fitness measures. For each individual, we calculated the average age of male and female social partners, weighted by the number of interactions with each partner.

The set of all interactions within each population was converted to a weighted, unidirectional social network. For each possible pair of individuals within the population, we calculated the simple ratio index, which is defined as the proportion of all survey periods at least one of the pair was observed that they were observed together (Ginsberg and Young 1992). We then calculated how socially connected each individual was using the network metric *strength*, calculated in the package tnet (Opsahl 2009). Strength measures an individual’s weighted number of interactions and can be tuned to adjust how repeated interactions with the same partner are weighted; we used a tuning parameter of 0.5, which means that the first interaction with a new partner adds 1 unit to strength and subsequent interactions 0.5 units (Opsahl 2009). Network strength in both-sex networks is repeatable in this species (Formica et al. 2017; Cook et al 2022), and selection on strength is highly variable among subpopulations in the wild (Formica et al. 2020).

Twenty-three beetles died over the course of the experiment. These individuals were included in the measurement of social neighborhoods and networks but were removed from all following analyses. The same dataset was used for a previous analysis of age and social behavior (Cook Chapter 2).

Statistical Analysis

We modeled reproductive success using two generalized linear mixed models, one for males and one for females. The sexes were analyzed separately because their reproductive success is estimated with different data—counts of mate-guards, which indicate successful inseminations, for males and counts of oviposition behaviours, which indicate the laying of a single egg, for females. Additionally, past work has shown that selection is often sex-specific (Costello et al. n.d.). Relative reproductive success was calculated locally in each population because social network phenotypes experience soft selection (Formica et al. 2020). These models included two covariates known to explain fitness variation in this species, the number of survey periods an individual was active and body size. Body size was estimated as the length of the elytra, measured in ImageJ (Abramoff et al. 2003), and was globally standardized because body size is under hard selection (Formica et al. 2020).

Past work has found that reproductive success varies across age cohorts, so we included individual age in these models as a categorical fixed effect. To assess the impact of social partner age on fitness, we added the average age of all males with whom each individual directly interacted to both models. Male partners influence male fitness through competition (Formica et al. 2011), and indirect evidence suggests that interactions with males are correlated with female laying (Costello et al. n.d.). To test whether population age structure impacts individual fitness, we added the age structure (either young or old) of the population, with the identity of the replicate population included as a random effect.

In addition to asking whether population age structure predicts the number of times we observed females laying eggs, we also looked at whether population age structure affected total reproductive output with a separate analysis. We counted the total number of egg scars on the surface of the fungus brackets in each population. An egg scar is the distinctive, long-lasting mark created when a female covers an egg with frass. These eggs cannot be attributed to specific mothers but include the lays that happened outside of our survey periods. A simple comparison of the total number of all eggs laid between the young and old populations was used to check that the observed behavioral data were a representative sample of all fitness events.

The last term in our mixed models tested whether selection on sociality, measured as network strength, differed between young and old populations. We included an interaction between standardized strength and population age structure. We know from past work that this design has sufficient power to detect differences in selection regimes between two conditions (Costello 2020).

Counts of successful inseminations (for males) and number of eggs laid (for females) were modeled assuming a Tweedie error distribution in the R package glmmTMB (Brooks et al. 2017; Dunn 2017) and model assumptions were checked visually and statistically using dHARMA (Hartig 2021). Marginal means were calculated in emmeans (Lenth, 2018). Because social network data violate the assumptions of conventional statistical tests, we assessed the significance of these models using a permutation approach (Farine 2017; Farine and Carter 2021). We used node rather than datastream permutations to avoid creating permuted datasets with different variances and inflating our false positive rate (Weiss et al. 2020; Cook et al. 2022). Each permutation randomized all variables used in the linear mixed models without replacement across individuals, breaking any relationships between those variables and fitness measures. We repeated this process 2000 times and ran the mixed models in each of these permuted datasets. The statistical significance of our observed estimates was determined by comparison to the distribution of estimates in the permuted datasets. P values were calculated as the proportion of all estimates greater than the observed estimate. All analyses were performed in R 4.1 (R Core Team 2021).

Results

Individual age, partner age, and group age structure all explained variation in reproductive success among males (Table 1). Both two- and three-year males were observed mate guarding females significantly more often than were one-year-olds (Figure 1A), but three-year-olds had lower fitness than two-year-olds males (posthoc pairwise contrast t = 2.99, p = 0.01). Males with higher average social partner age had fewer successful inseminations than males who interacted with younger males (Figure 1B). Overall population age structure also accounted for some variation in male fitness; males in the young populations had higher average fitness than did males in old populations (Figure 1C). Male body size and network strength were both under positive selection, and selection on social network position did not differ with population age structure (Figure 1D). Activity level was not associated with male fitness.

Raw counts of all eggs showed that old populations produced on average 38 more eggs (or approximately 2 more eggs per female) than did young populations over the three weeks of the experiment (95% CI = 14.5 – 60.7). The mixed model results found that individual age and population age explained variation in female fitness, but the average age of male partners did not (Table 2). Two- and three-year-old females laid more eggs than did one-year-olds (Figure 2A), and females in young populations laid more eggs than females in old populations (Figure 2B). Neither activity level nor body size predicted female fitness, but network strength was under positive selection. The interaction between network position and population age structure shows that individual strength was only under selection in the old populations (Figure 2D).

Discussion

Our results show that age is an evolutionarily significant component of the social environment for *B. cornutus*, impacting both individual fitness and its relationship with social network phenotypes. Variance in age is associated with fitness across three different levels of organization: the individual, social group, and population. Older members of both sexes have higher reproductive success than tenerals; males, but not females, pay a fitness cost to interacting with older male social partners; and both sexes have lower fitness in groups with older age compositions. Selection on social network position in females depends on the age structure of the population. Our results suggest that differences the age of the social environment, within or among populations, are a source of variation in both fitness and selection pressures.

Group age structure changes sexual selection regimes

Population age composition altered sexual selection gradients on female network sociality in both-sex networks. The network strength of females was not correlated with fitness in young populations, but experienced positive directional selection in old populations. It is possible that being more connected is only beneficial for females in sparser networks, and not in the dense networks formed by young populations (Cook Chapter 2). However, the exact mechanisms of selection of social network position are still unknown. Understanding how selection gradients vary across multiple demographic variables will help illuminate possible mechanisms (Formica et al. 2020; Costello et al. n.d.). Importantly, we find that although age covaries with multiple fitness-relevant traits, including strength (Cook Chapter 2), selection on strength in both sexes remains significant even after controlling for age at multiple levels of social organization. Moreover, adding this previously overlooked axis of variation at the group level has revealed a demographic factor which may contribute to variation in sexual selection across a metapopulation. In the wild, age structure may covary with the time elapsed since population founding as well as genetic relatedness (Whitlock 1992), and relatedness may impact selection on social behavior (Rodrigues 2018). However, because the populations in this study were randomly assembled from a breeding colony, our experimental design avoids these confounds. Age structure, independent of other demographic factors, contributed to variation in selection gradients among our populations. This is, to our knowledge, a novel addition to the body of existing work on the complex relationship between age and sexual selection (Bonduriansky et al. 2008; Roach and Carey 2014).

Our results suggest that age structure may contribute to spatial and temporal variation in the selective landscape. Selection is a powerful evolutionary force, but we understand fairly little about its ecological drivers (Morrissey and Hadfield 2012; Siepielski et al. 2017). We suggest that further work consider age structure as a factor shaping selection regimes, especially on social behaviors such as social network traits. As interacting phenotypes, social behaviors may be both more likely than other traits to experience variable selection and more likely to respond rapidly (Moore et al. 1997; Wolf et al. 1998; Formica et al. 2020).

Individual, partner, and group age impact fitness

At the individual level, we find that older males and females have significantly higher reproductive success than tenerals. One possibility is that these patterns are caused by individuals increasing their reproductive success over their lifetimes. Because this species is holometabolous, such improvement could not be due to increased size, but instead might be related to energy storage, higher aggression, more experience with competition or courtship behaviors, and preferences among members of the other sex. The overall increase in fitness is consistent with an early study in this system which found that males increased their rates of insemination success over the first 60 days since initial sighting (Conner 1989b). However, disentangling change over time from selective disappearance will require longitudinal datasets following the same beetles over multiple years. Another question for future work would be whether the sexes differ in rates of reproductive senescence—our data show females of the oldest age class laying as many eggs as two-year-olds, but the oldest males mate-guarding less often than two-year-olds. Other studies of wild invertebrates have found faster senescence of males than females (Rodríguez-Muñoz et al. 2019; Zajitschek et al. 2020), possibly because males invest energy early on in their lives to be competitive (Bonduriansky et al. 2008). But most studies of reproductive senescence in the wild have focused on females, and more studies are needed to understand patterns of male aging (Lemaître and Gaillard 2017). Another possibility for further work to evaluate is whether selective pressures depend on individual age (Coltman et al. 2002; Martin et al. 2016; Turner et al. 2020).

Male fitness was also negatively correlated with the age of social partners. This may be a sign of a more challenging competitive environment, since age is positively associated with aggression (Mitchem et al. Submitted) and males have lower mating success when surrounded by high-quality competitors (Formica et al. 2011). Spatially varying age social environments may therefore create variation in the competitive environment within a single population. Because individual and partner age are correlated (Cook Chapter 2), this effect will counter the fitness advantage of older males. By contrast, we found no cost to females of interacting with older male social partners, despite evidence that female fitness is also negatively impacted by high numbers of interactions and courtship attempts from males (Costello et al. n.d.). One explanation for this lack of observed effects is that that the number of interactions is what matters for female fitness, rather than the characteristics of the partner. Another is that that older males are more aggressive with other males but not with potential males; within individuals, aggression in intrasexual competition is not correlated with behaviours in intersexual mating interactions (Mitchem 2021).

At the population level, counts of total eggs show that populations of older beetles produced more potential offspring than young populations, consistent with finding that older females lay more eggs than young females. However, after controlling for individual age, our modeling results find that both sexes achieve lower reproductive success when surrounded by old conspecifics. The apparent contradiction highlights an important reason for considering multiple levels of selection: membership in a group with higher total reproductive output can lower relative fitness. The potential benefits of being surrounded by members of the opposite sex heavily invested in reproduction are outweighed by high average fitness in these conditions.

Natural history considerations

The wide range of ages within our experimental populations, from beetles eclosed only two weeks before the study began to those at least four years old, was possible because of the surprisingly long lifespan of forked fungus beetles. Although social insect queens can live and reproduce for many years (Keller and Genoud 1997), there are few insect species in which a large fraction or even the majority of residents in population could be mature adults more than one year old. Iteroparity may be more common among Coleoptera than other orders, but only a handful of such cases have been documented (reviewed in Pace 1967b pp. 6–7; Danks 1992 p. 178; Promislow et al. 2022 Figure 1), and of these *B. cornutus* appears to be one of the most extreme, with some individuals observed mating and ovipositing across five breeding seasons

(Formica, personal communication). However, there are relatively few long-term studies of wild insect populations. There may be more insects with long life cycles than are currently recognized, as measuring invertebrate age and lifespan can be difficult (Danks 2000). The conditions favoring such strategies seem to include cool and stable climates; reliable resource availability over the winter, to increase the odds of surviving to the next breeding season and therefore the fitness payoff of extend lifespan; and low-quality food that is difficult to digest (Pace 1967b; Danks 1992; Gillott 2005; Slipinski and Lawrence 2013). Thick cuticles may also be associated with longer lifespans in insects (Gillott 2005). Long-term studies of insect species meeting these criteria may reveal more examples of extended reproductive windows.

However, we emphasize that no one life history strategy is required to produce the patterns seen in this study. Similar results might be found in any taxon which meets two requirements. First, age structure must vary at the level of populations or subpopulations, whether because of differences in the time since founding, histories of disturbance, or predation regimes. Second, individual age must be associated with a trait likely to affect conspecific fitness, such as aggression. Such an association might be created through senescence, through learning or other forms of improvement over time, or by natural selection acting within a generation such that individuals surviving to old age represent a non-random sample of the initial cohort.

Acknowledgments

We thank Savanna Cabrera, Hannah Donald, and Sarah McPeek for help with data collection; Savanna Cabrera and Ahema Gaisie for data entry; and Alejandro Medina-Valencia for data cleanup. The staff of Mountain Lake Biological Station provided advice, support, and resources. Funding for this work was provided by an NSF Graduate Research Fellowship for P.A.C., a

University of Virginia Double Hoo research award to P.A.C., NSF IOS-1355029 to V.A.F. and IOS-1355003 and DEB-1911485 to E.D.B.III, and NSF REU grant 1461169 to Mountain Lake Biological Station.

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TABLES AND FIGURES

Table 1. Estimates and significance values of all fixed effects in the generalized linear mixed models predicting male reproductive success. Model estimates (slopes) are reported for continuous variables, back-transformed marginal means for categorical variables, and back-transformed marginal trends for the interaction. P-values are calculated as the proportion of 2000 permuted model F-statistics that were greater than the observed model estimate. P-values significant at the α = 0.05 level are in bold.

	Estimate	P-value
Scans active	0.01	0.394
Body size (mm)	0.26	**<0.01**
Individual age	one: 0.22 two: 1.43 three+: 0.83	**<0.01**
Male social partner age	-0.14	**0.02**
Population age structure	young: 0.76 old: 0.55	**0.017**
Network strength	0.30	**<0.01**
Age structure x strength	young: 0.31 old: 0.30	0.927

Table 2. Estimates and significance values of all fixed effects in the generalized linear mixed models predicting female reproductive success. Model estimates are reported for continuous variables, back-transformed marginal means for categorical variables, and back-transformed marginal trends for the interaction. P-values are calculated as the proportion of 2000 permuted model F-statistics that were greater than the observed model estimate. P-values significant at the α = 0.05 level are in bold.

	Estimate	P-value
Scans active	0.01	0.628
Body size (mm)	-0.12	0.155
Individual age	one: 0.29 two: 1.30 three+: 1.12	**<0.01**
Male social partner age	-0.14	0.232
Population age structure	young: 1.01 old: 0.55	**0.001**
Network strength	0.17	**0.037**
Age structure x strength	young : 0.00 old: 0.34	**0.038**

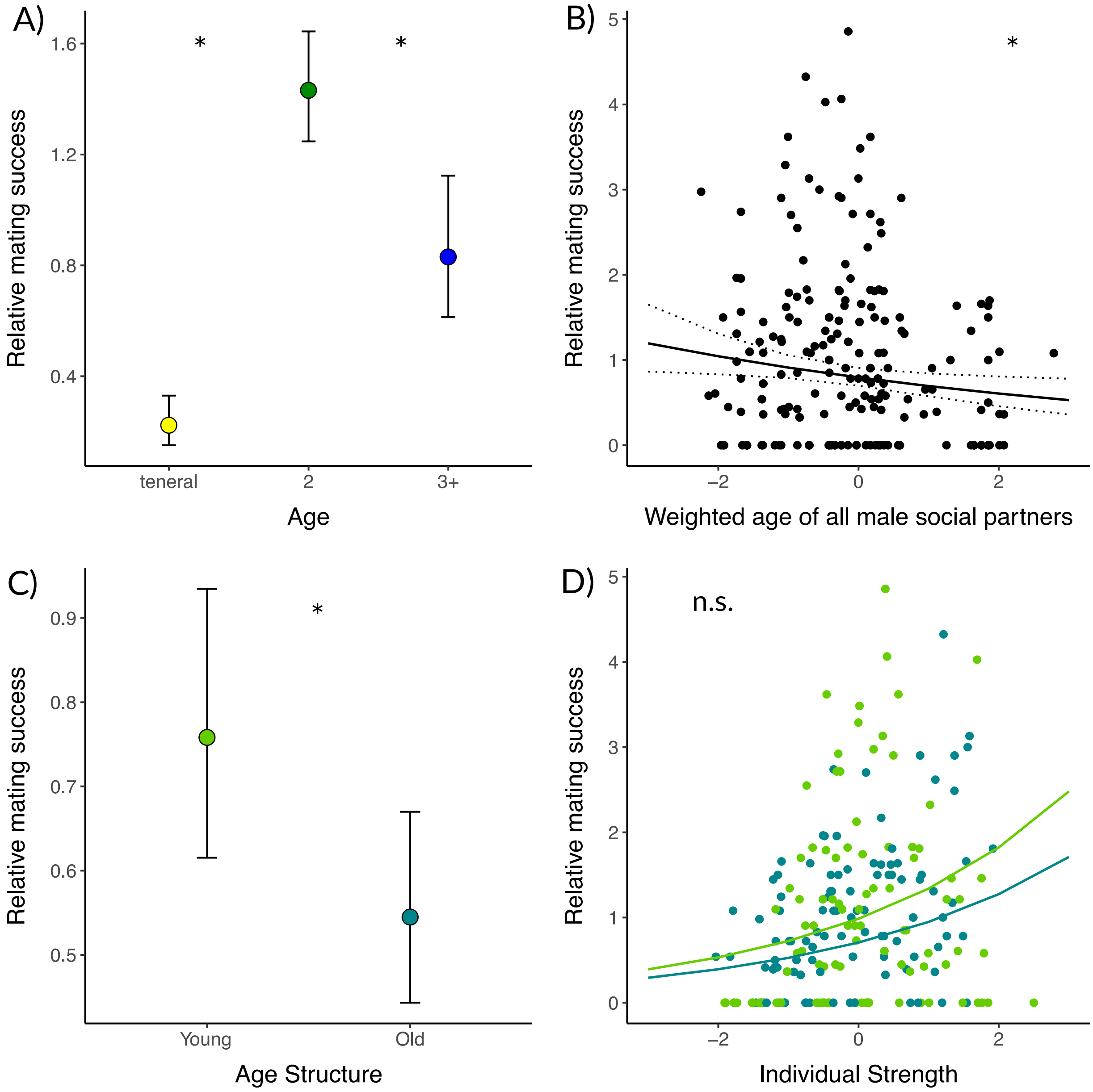

Figure 1. Selected fixed effects from the model of male reproductive success, measured as the number of mate guarding events observed for that individual relative to other members of its population A) Back-transformed marginal means of reproductive success for the three age cohorts. B) Marginal effects of average male partner age on male fitness, with raw data plotted. C) Back-transformed marginal means of male reproductive success in the two age structure treatments. Note that this is a main effect involved in an interaction. D) Marginal effects of individual network strength on male fitness, subset by population age structure. Lighter points represent raw data from young populations, and darker points denote old populations. Selection on male strength is positive and similar in magnitude in both treatments.

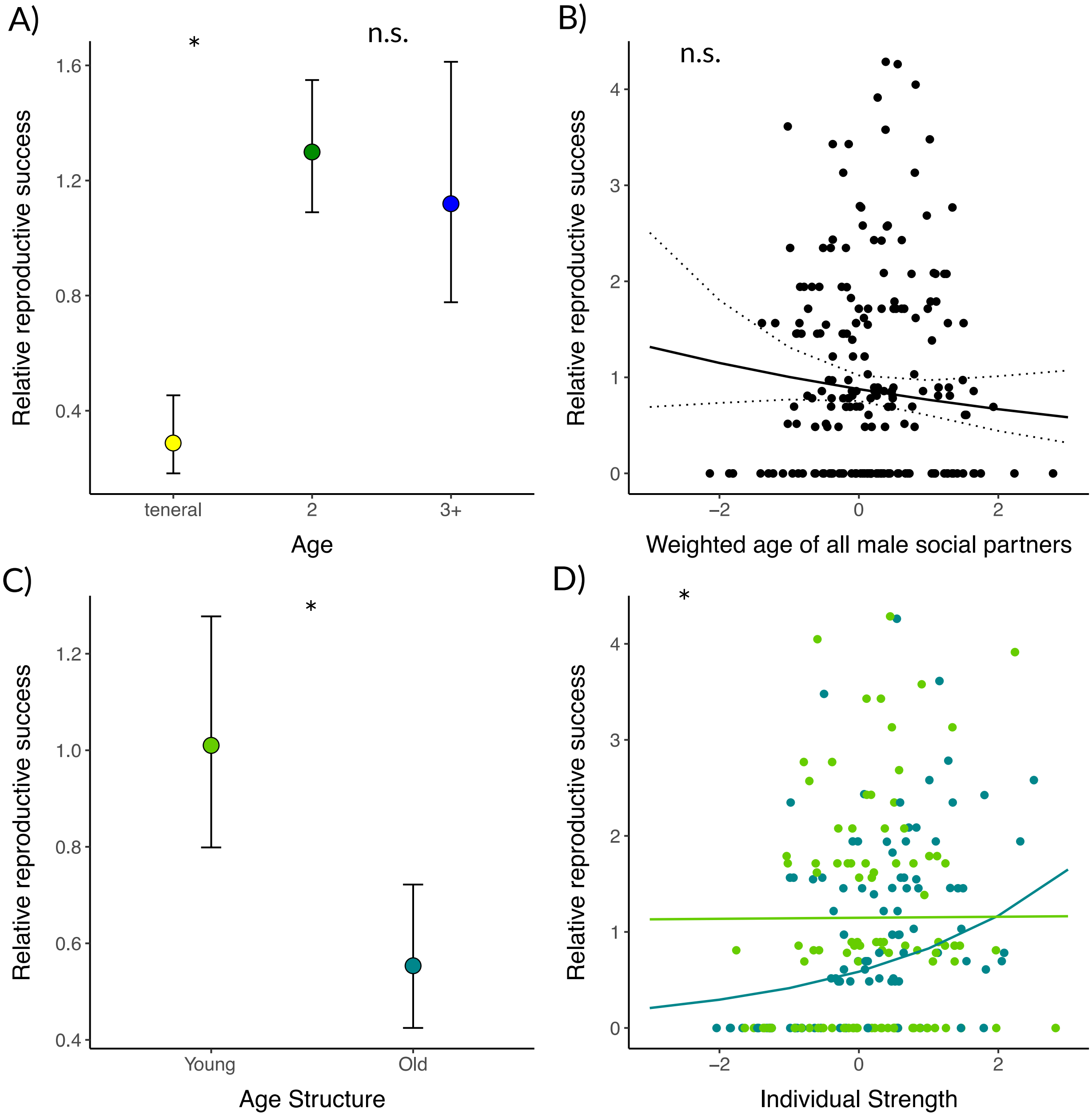

Figure 2. Selected fixed effects from the model of female reproductive success, measured as the number of oviposition events observed for that individual relative to other members of its population. A) Back-transformed marginal means of reproductive success for the three age cohorts. B) Marginal effects of average male partner age on female fitness, with raw data plotted. C) Back-transformed marginal means of female reproductive success in the two age structure treatments. Note that this is a main effect involved in an interaction. D) Marginal effects of individual network strength on female fitness, subset by population age structure. Lighter points represent raw data from young populations, and darker points denote old populations. Selection regimes differ between populations with young and old age structures.