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Original Article

Social behavior and activity are decoupled in larval and adult fruit flies

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The growing body of literature on social behavior in fruit flies opens up exciting opportunities for addressing an unresolved issue involving the degree of correlation between behavioral traits in larvae and adults. Although the prevailing adaptive decoupling hypothesis states that metamorphosis is associated with the disruption of genetic correlations between juvenile and adult traits, 2 alternative hypotheses are that, sometimes, a positive correlation may be adaptive, and that, often, the underlying genetic architecture will prevent perfect decoupling. We used lines of the *Drosophila* Genetic Reference Panel to quantify the degree of sociality in larval and adult fruit flies and then examined the correlation between the life stages. To verify that our social behavior scores did not merely reflect variation in activity levels, we also quantified larval and adult activity. Although we found significant variation in social behavior and activity among larvae and adults, both traits were decoupled between larvae and adults. Social behavior and activity were not positively correlated within each life stage either. Although our results agree with the adaptive decoupling hypothesis, both ultimate and proximate considerations suggest that, generally, we should expect the degree of decoupling to vary between species and traits.

Key words: **activity, *Drosophila melanogaster*, fruit flies, metamorphosis, social behavior.**

INTRODUCTION

Complex life cycles involving metamorphosis from larvae to adults occur in the majority of animal phyla and about 80% of animal species (Werner 1988). The adaptive decoupling hypothesis, made perhaps first by Haldane (1932), explains the prevalence of complex life cycles by stating that antagonistic selection leads to the disruption of genetic correlations between juvenile and adult traits, which can then evolve independently via distinct developmental programs (Ebenman 1992; Moran 1994). Although the adaptive decoupling hypothesis is intuitively appealing and obviously agrees with the fact that larvae and adults are anatomically and morphologically distinct, its relevance to behavior is not clear. Theoretically, one can readily think of 2 equally attractive alternatives. First, at the ultimate level, it may actually be adaptive for individuals to maintain similar behavioral phenotypes across metamorphosis. In this case, selection on a certain behavioral trait in larvae and adults will be facilitatory rather than antagonistic. Second, at the proximate level, it is possible that the complex genetic architecture that determines certain behaviors is resistant to decoupling without negatively influencing the life stage where it contributes most to fitness (Arnold 1990; Marshall and Morgan 2011; Aguirre et al. 2014).

The limited empirical data on larval–adult genetic correlations in behavior indeed provide no support for the adaptive decoupling hypothesis. In tree frogs (*Hyla regilla*), larval and adult phases showed positive genetic correlation in locomotor traits, perhaps because these were correlated with measures of body size, which were positively associated between phases (Watkins 2001). Similarly, even though Wilson and Krause (2012b) predicted no correlation in behavioral traits between tadpoles and juvenile lake frogs (*Rana ridibunda*) owing to their distinct ecologies, they actually documented a positive correlation in activity and exploration between the life stages. In damselflies (*Lestes congener*), which undergo a transition similar to that of frogs from aquatic to terrestrial life, activity and boldness were positively correlated between larvae and adults (Brodin 2009). Finally, perhaps the only relevant work in fruit flies (*Drosophila melanogaster*) suggests a positive genetic correlation in behavior between larvae and adults. The naturally occurring genetic polymorphism in the *for* gene affects levels of exploration in the larvae (Sokolowski 1980; Osborne et al. 1997). Recent experiments indicate that the same *for* alleles also determine parallel adult morphs with distinct tendencies to explore (Edelsparre et al. 2014). Data on larval–adult genetic correlations in nonbehavioral traits do not provide strong support for the adaptive decoupling hypothesis either, with some agreeing with the prediction whereas others indicating significant genetic correlations between larval and adult traits (Loeschcke and Krebs 1996; Parichy 1998; Crean et al. 2011; Fellous and Lazzaro 2011).

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Our focus is on the correlation in social behavior across metamorphosis, a topic that, as far as we know, has not been addressed previously. Our study species is fruit flies, which are highly attractive owing to the numerous tools available for mechanistic and evolutionary research in this species. It has been known for a long time that adult fruit flies' aggregation is mediated by attraction to cis-vaccenyl acetate, which males transfer to females during copulation (Brieger and Butterworth 1970; Bartelt et al. 1985; Wertheim et al. 2002). Recent research also indicates strong adult attraction to volatiles emitted by larvae (Durisko, Anderson, et al. 2014; Venu et al. 2014). Furthermore, social interactions synchronize flies' circadian clock (Levine et al. 2002), influence decisions about egg laying (Sarin and Dukas 2009; Battesti et al. 2012) and mating (Krupp et al. 2008; Mery et al. 2009; Billeter et al. 2012), and the composition of cuticular hydrocarbons (Kent et al. 2008; Krupp et al. 2008).

Although much of the research on fruit fly social behavior has focused on the adult stage, recent work indicates elaborate social interactions among larvae as well. The larvae show strong social attraction to other larvae and learn to prefer odors previously associated with other larvae (Durisko and Dukas 2013). Newly hatched larvae actively seek each other and form social aggregations, which enhance their ability to burrow into fruit (Durisko, Kemp, et al. 2014). Finally, wandering third instar larvae rely on odor cues to form conspecific pupal aggregations (Beltramí et al. 2010; Del Pino et al. 2014). The detailed data on social behavior in larval and adult fruit flies open up exciting opportunities for examining the magnitude of genetic correlation in social behavior across metamorphosis as well as its mechanistic and functional bases.

As with any other trait, one can predict adaptive decoupling of social behavior given the distinct ecologies of larvae and adults. One can, however, also predict adaptive coupling. For example, assuming that larval densities are highly positively correlated with adult densities, if a tendency to form social groups is beneficial under both high larval densities and high adult densities, it can be adaptive for fruit flies to possess a tight positive genetic correlation between larval and adult social behavior. Finally, one can also predict coupling owing to genetic constraints. Indeed the limited literature on larval–adult correlations detailed above suggests that coupling of behavioral and other traits, with no apparently adaptive explanation, does exist in a few of the species studied.

To assess the magnitude of genetic correlation in social behavior between larval and adult fruit flies, we modified a protocol used to quantify spontaneous social behavior in the larvae (Durisko, Kemp, et al. 2014) to measure the tendency to form social groups in both larvae and adults of isofemale lines of the *Drosophila* Genetic Reference Panel (DGRP; Mackay et al. 2012). Because the DGRP lines are fully sequenced, our methods and results provide a solid foundation for further research on the genetic architecture underlying social behavior across life stages, which will likely be relevant to many animals given the remarkable genetic similarity among species (Inlow and Restifo 2004; Bolduc and Tully 2009; van Alphen and van Swinderen 2013; Rodriguez et al. 2013). Although our major goal was to quantify the correlation in social behavior between larvae and adults, we also wished to verify that social behavior is not merely a by-product of activity levels. We thus also independently assessed activity levels of individual larvae and adults. These data also allowed us to test for the correlation between activity in larvae and adults, which is of broad interest given that activity has been measured in a large variety of species in the context of behavioral syndromes (e.g., Watkins 2001; Brodin 2009; Wilson and Krause 2012a; Sih et al. 2014). Overall then, we addressed 5 questions. First, is there significant genetic variation in social behavior in larval and adult fruit flies? Second, is there significant genetic variation in activity in larval and adult fruit flies? Third, is social behavior positively correlated with activity levels within larvae and within adults? Fourth, is there a positive correlation in social behavior between larvae and adults? Finally, is there a positive correlation in activity between larvae and adults? Our questions are relevant for the majority of animals, which undergo metamorphosis, as well as for research addressing behavioral syndromes throughout animal lives in other taxa including lizards, birds, and mammals (Arnold 1990; Class and Brommer 2015).

METHODS

General

We used 29 lines of fruit flies belonging to the DGRP (Mackay et al. 2012). These isofemale lines were established from wild-type gravid females from the Raleigh, North Caroline farmer's market in 2003, and inbred by 20 generations of full sibling mating, followed by random mating. Although we initiated our work with 40 lines, we narrowed our focus to the 30 most robust lines and later lost 1 line. We maintained flies at low densities in vials containing 5 mL of standard food (1 L of which contained 90-g sucrose, 75-g cornmeal, 32-g yeast, 16-g agar, and 2-g methyl paraben) at 25 °C and 60% relative humidity on a 12:12 light cycle with lights off at 10 PM. We conducted egg laying for experimental adults in vials containing standard food and a sprinkle of live yeast. For our larval experiments, we reared parental adult flies on an altered light cycle with lights off at 1 PM, which placed peak egg laying at midday. We collected eggs for experimental larvae in food vials without added live yeast between 1 and 3 PM to minimize hatching asynchronies. We removed excess eggs from the surface of all egg laying vials to maintain similar densities across all vials.

To eliminate bias, we conducted all the data recording while being blind to fly line. For video analysis, we used Python 2.7 (Python Software Foundation 2015) and OpenCV 2.4.11 (OpenCV 2015). We analyzed all data in R version 3.2 (R Core Team 2014) using general linear mixed-effect models (Bates et al. 2014) and parametric bootstrapping (with 1000 iterations) to test all relevant random effects (R package pbkrtest; Halekoh and Højsgaard 2014). We report Wald χ^2 for all fixed effects, and bootstrapped P values for all tested random effects. To test for all correlations, both within and between life stages, we performed linear regressions. Finally, we calculated broad sense heritability (H^2) as the line variance/(line + residual variance) (Falconer and Mackay 1996; Shorter et al. 2015).

Larval social behavior

One day after collecting eggs for experimental larvae, we placed 12 recently hatched larvae of the same line in the center of each 35-mm Petri dish containing standard food, colored blue to improve visibility. We stored dishes in complete darkness at 25 °C and high (>75%) humidity for the duration of the experiment. As a compromise between reducing temporal variation and potentially interfering with larval behavior, we observed each group of larvae under red light (650 nm) at both 44- and 52-h posthatching (8 AM and 4 PM), 2 time points corresponding to the late 2nd instar.

During observations, we overlaid a transparent 0.1 cm^2 grid across the top of the dish, and marked the locations of the larvae on 1:1 scale grid paper. We then scanned the observation sheets

and obtained the Cartesian coordinates of larvae using ImageJ (Schneider, Rasband, et al. 2012). We used the distances between larvae to calculate a nearest neighbor index for each dish. The nearest neighbor index is a measure of distribution in space, defined by the ratio between mean observed nearest neighbor distance and that expected by random chance at the given density. Nearest neighbor indices range from 0, where all points occupy the same region in space, to 2.15, representing a perfectly uniform distribution (Clark and Evans 1954; Krebs 1999). That is, highly social larvae that form a tight group will have a small nearest neighbor index, whereas nonsocial larvae that avoid each other will have a large nearest neighbor index. Preliminary analyses indicated that nearest neighbor indices were normally distributed. With a few exceptions owing to insufficient number of hatchlings, we tested 30 dishes of larvae, up to 15 per day, from each genotype. We excluded from our analysis dishes in which we found fewer than 8 larvae during either of our observations due to mortality, escape from the dish, or burrowing. In total, we tested 851 groups of larvae across 10 days. Our model included nearest neighbor index as a dependent measure, time of observation as a repeated measure, and genotype, day, and dish as random effects.

To assess the repeatability of our protocol in quantifying social behavior, we performed a second test on larvae from the 6 genotypes observed to have the lowest mean nearest neighbor indices and the 6 genotypes with the highest mean nearest neighbor indices during our first test. We tested up to 25 dishes of larvae from each of the 12 lines, for a total of 281 dishes over 2 days. We analyzed the data using a general linear mixed-effect model, which included group (high or low social) and time of observation as fixed factors, and genotype, day, and dish as random effects. We predicted that larvae from the 6 lines identified in the first screening as being more social would once again show lower mean nearest neighbor indices than larvae from the 6 lines previously identified as being less social.

Larval activity

At 1 PM on the day following egg laying, we transferred groups of 20 recently hatched larvae from each of the 29 DGRP lines into 35-mm Petri dishes containing 8 mL of standard food so that larvae developed at identical densities. We maintained experimental larvae at high (75%) humidity, 25 °C, and on a 12-h photoperiod (with lights on at 1 AM) until observations. We conducted all observations between 8:30 AM (approximately 44-h posthatch) and 12:30 PM. We transferred larvae individually into 35-mm experimental Petri dishes containing standard food (colored black to improve visibility) and placed up to 10 dishes at a time into one of 2 boxes (53 cm × 31 cm × 30 cm; length × width × height). The boxes were uniformly illuminated by two 3-W LED bulbs suspended 37 cm above the dishes on opposite sides of the box lid (35.5 cm apart). Five minutes after placing the larvae in the boxes, we video recorded them with high-resolution webcams (Logitech C920) through a hole in the center of each box lid. Video recording lasted 10 min, a duration long enough to capture movement, but short enough to minimize larval burrowing into the food medium.

We integrated video frames over 0.5-s time windows to reduce pixel variation prior to analysis, which consisted of calculating the centroid coordinates of larvae at each time point, and partitioning the cumulative distance traveled by each larva into 2-min bins. To reduce noise, movement occurrences were only scored if the larvae had moved more than 0.5 mm from their previously recorded locations. We verified all automated analysis in real time while being blind to larval line. We omitted data from 24 larvae that burrowed into the medium during the 10 min of observation. In total, we collected data from 497 larvae over a span of 2 days. We analyzed the data with a general linear mixed-effect model with time of day and day of observation as fixed effects, elapsed time as a repeated measure, and genotype, dish, and box all as random effects. We evaluated the genotype contribution using parametric bootstrapping.

Adult social behavior

We collected experimental adults within 6 h of eclosion, sexed them under light CO_2 anesthetic, and placed them into mixed-sex vials containing 14 males and 14 females. Approximately 70-h posteclosion, at 9 am, we briefly anesthetized experimental adults and placed groups of 12 single-sex adults, either males or females, one group inside each 35-mm experimental Petri dish. These dishes contained 8 mL of standard food, with corn meal omitted to reduce the heterogeneity of the food surface texture. The volume of food in each dish was sufficient to minimize headspace, effectively constraining flies to 2 dimensions. We left flies to acclimatize for 5 h, after which we placed up to 10 dishes into each of 6 boxes equipped with webcams identical to the boxes used in the larval activity experiment. We allowed flies to acclimatize for 30 min and then video recorded them for 30 min. We conducted all observations during one of 2 test sessions, beginning at either 3:00 or 3:30 PM, with no more than a single group of males and females from a single line being tested on the same day. In total, we collected data from 823 dishes of flies, observed across 26 days.

Video analysis consisted of sampling single frames at 30-s intervals, determining the Cartesian coordinates of each fly's centroid, and calculating nearest neighbor distances. Because the number of visible individuals was always 12, we used the median nearest neighbor distance as a measure of spatial distribution rather than computing a nearest neighbor index. The former measure was less variable than the index because the average nearest neighbor measured for the index was strongly influenced by highly mobile outlying individuals over short time scales. We corrected all automated video analyses while being blind to fly line. To correct for body size differences between the sexes, we measured the body length (anterior antennae to posterior abdomen) and thorax width of a subset of 20 males and 20 females, calculated the diameter of an equivalent sized circle, and subtracted this value from the median nearest neighbor distances of both sexes (1.63 and 1.85 mm for males and females, respectively).

We analyzed the data using a general linear mixed-effects model with sex, time, Wolbachia infection status, and test session as fixed effects. We added the Wolbachia as an independent factor because recent data indicated that about half the DGRP lines are infected by this bacterium (Huang et al. 2014), which may influence levels of aggression (Rohrscheib et al. 2015). The Wolbachia infection status for each DGRP line is reported by Huang et al. (2014). We included day, box, and dish as simple scalar random effects and a random effect of sex, varied by genotype, in our full model. We then constructed 2 nested models in which sex was either reduced to a simple scalar random effect of genotype or omitted entirely. These nested models were sequentially compared using parametric bootstrapping to test both the significance of the genotype by sex interaction and the main effect of genotype.

Adult activity

Concurrent with our observations of adult social behavior, we assayed the 29 DGRP lines for levels of activity, quantified as the walking path length of single flies. We transferred flies individually into 35-mm experimental Petri dishes. Four hours later, we placed

up to 10 dishes in each of 6 boxes and allowed flies to acclimatize for 30 min before video recording them for 30 min. We started all observations at either 1:30 or 2:00 PM, with a maximum of a single male and female from each line tested on the same day. Video analysis was identical to the previous experiment, with the exception that we partitioned data into 5 min bins to reduce the occurrence of zeros in the data set caused by minimally active flies.

We analyzed the data using a general linear mixed-effects model with sex, Wolbachia infection status, and test session as fixed effects and time as a repeated measure. As simple, scalar random effects, we included day, box, and dish. Similar to the analysis of adult social behavior, we included a random effect of sex varied by genotype in our full model, which was compared with 2 nested models in which this term was omitted or reduced to test for the effect of genotype and its interaction with sex, respectively.

RESULTS

Larval social behavior

Larval nearest neighbor indices varied significantly by larval genotype (range of mean nearest neighbor indices: 0.884–1.197; $P < 0.01$; Figure 1a), yet remained consistent between larvae tested at 44- and 52-h posthatch (Wald $\chi^2_1 = 1.88$, $P = 0.17$). When comparing nearest neighbor indices between the first and second tests, the 6 lines initially identified as more social once again had significantly lower nearest neighbor indices than the 6 lines initially identified as less social (Wald $\chi^2_1 = 14.26$, $P < 0.001$; Figure 1b). Finally, the broad sense heritability of larval social behavior was 0.12.

Larval activity

A significant proportion of the variation in larval path length was explained by genotype ($P < 0.01$; Figure 1c). There was a weak but significant decline in path length over the 10-min test (Wald $\chi^2_1 = 7.8$, $P < 0.01$). Additionally, activity increased steadily across the 4 h of data recording, peaking before the start of the dark period (Wald $\chi^2_1 = 108.5$, $P < 0.001$). Finally, the broad sense heritability of larval activity was 0.42.

Correlation between larval activity and social behavior

Larval nearest neighbor indices were not significantly correlated with larval activity (linear regression; $F_{1,27} = 2.18$, $P = 0.15$; Figure 1d).

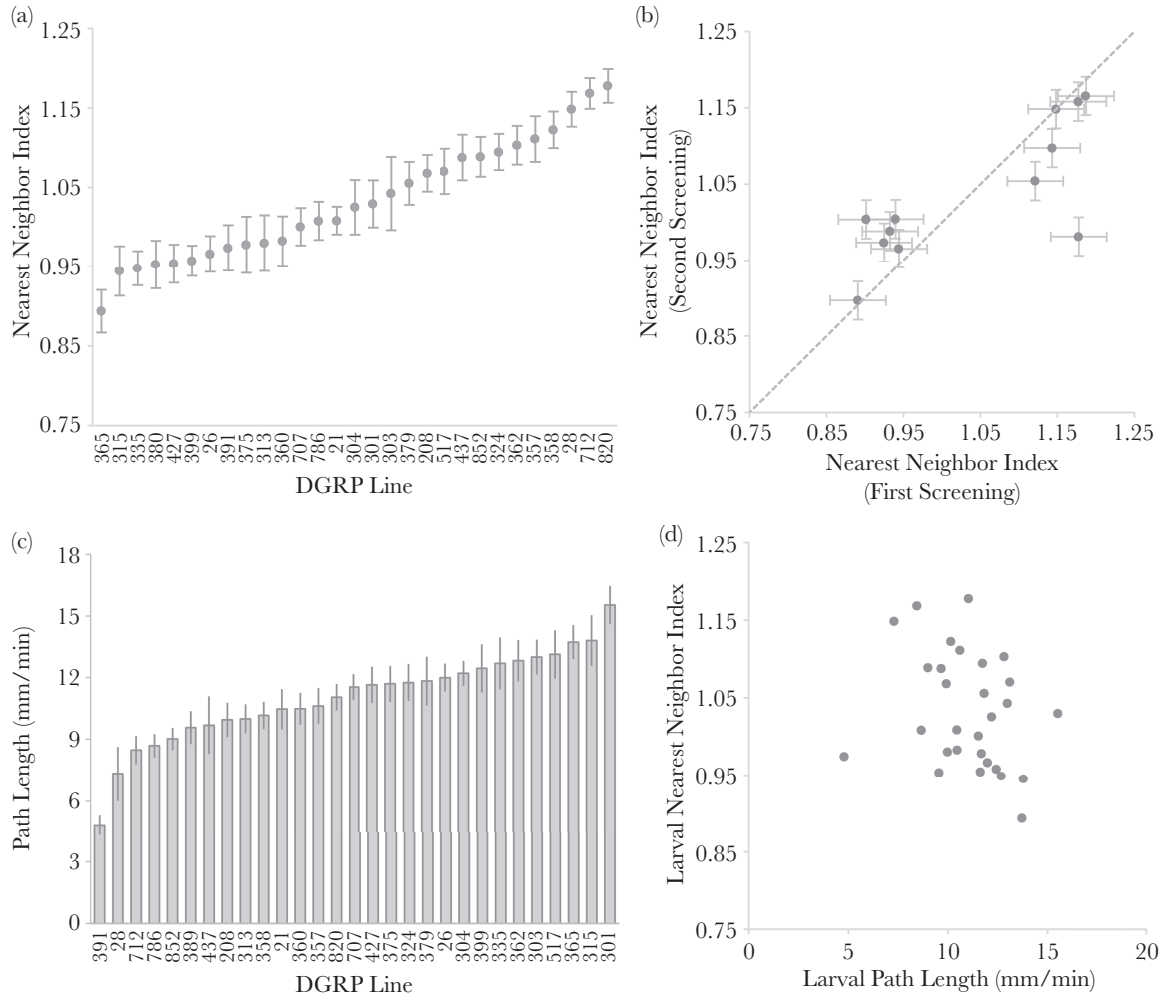

Figure 1

Larval data. (a) Mean ± standard error (SE) nearest neighbor indices of larvae from the 29 DGRP lines assayed ($N = 24$–55 replicates per line). The data from the 12 lines tested in both the first and second tests were pooled together. (b) Relationship between mean (± SE) nearest neighbor indices observed during the first and second tests for 12 DGRP lines. (c) Mean ± SE movement rate (millimeter/minute) of larvae among the 29 DGRP lines assayed ($N = 15$–18 larvae per line). (d) Relationship between mean movement rate and nearest neighbor indices for 29 DGRP lines tested.

Adult social behavior

Median nearest neighbor scores showed significant variation with respect to genotype ($P < 0.01$). Although there was no significant main effect of sex (Wald $\chi^2_1 = 2.91$, $P = 0.09$), the interaction with genotype was highly significant ($P < 0.001$; Figure 2a). There was no significant change over time (Wald $\chi^2_1 = 0.59$, $P = 0.44$) nor difference in scores with regards to either test session (Wald $\chi^2_1 = 0.49$,

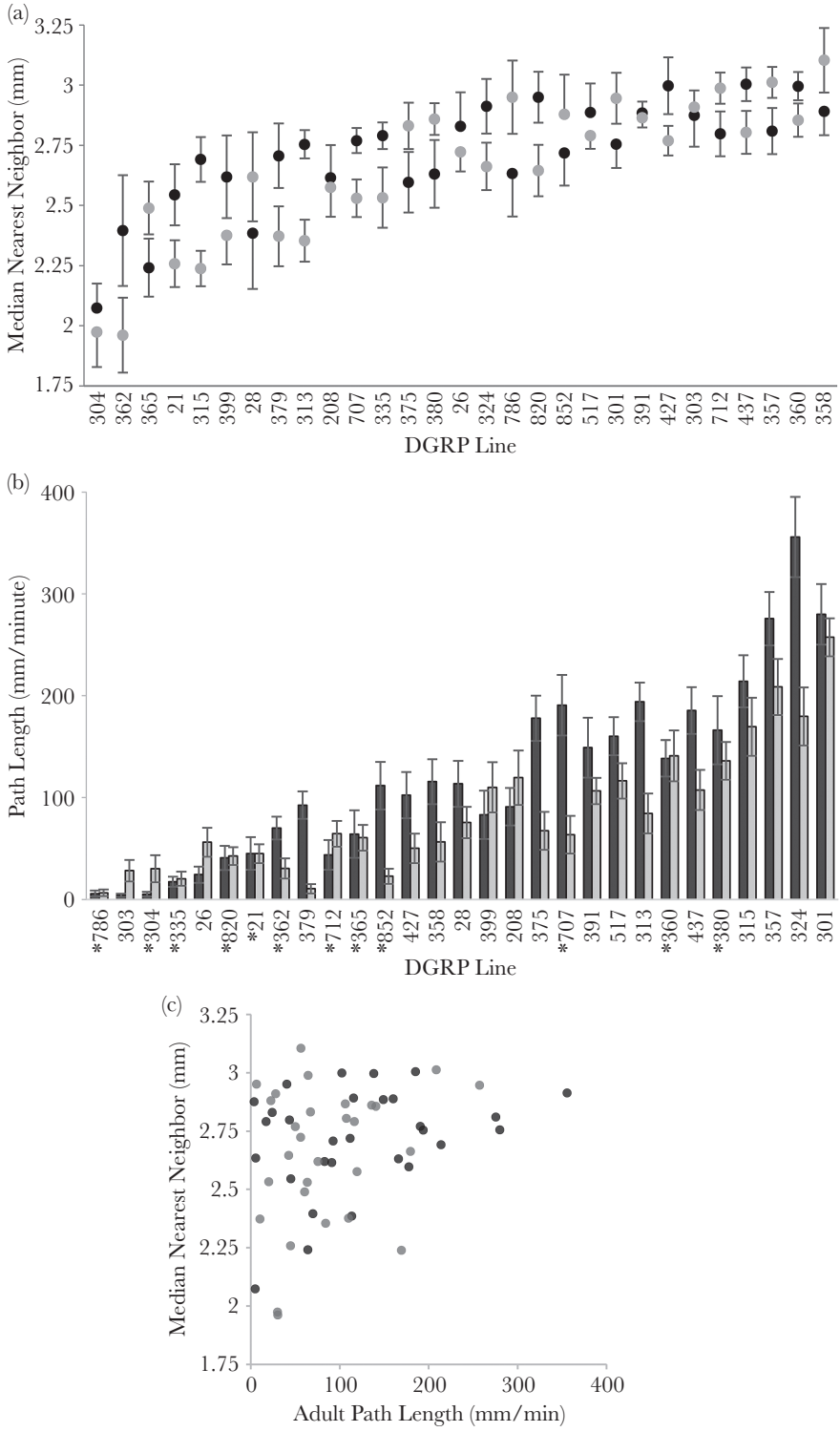

Figure 2

Adult data. (a) Mean ± standard error (SE) median nearest neighbor distances for groups of adult male (black) and female (gray) flies belonging to each of 29 DGRP lines assayed ($N = 9$–16 replicates per sex/line). (b) Mean ± SE movement rates amongst walking male (black) and female (gray) adults from the 29 DGRP lines tested ($N = 9$–16 per sex/line). Lines with known Wolbachia infection are marked with an asterisk (*). (c) Relationship between movement rates and nearest neighbor distances for male (black) and female (gray) adults belonging to each of 29 DGRP lines tested.

$P = 0.48$) or Wolbachia infection status (Wald $\chi^2_1 = 3.64$, $P = 0.06$). Finally, the broad sense heritability of adult social behavior was 0.04 in both males and females.

Adult activity

We found a significant main effect of genotype ($P < 0.01$) and sex (Wald $\chi^2_1 = 4.83$, $P < 0.05$), as well as a significant interaction between the two ($P < 0.01$; Figure 2b). Overall, males displayed more movement than females; however, the extent of this effect varied across the 29 lines. Despite 30 min of acclimatization before beginning data recording, there was a significant reduction in activity over time (Wald $\chi^2_1 = 226.09$, $P < 0.001$), though there were no differences in activity between the first and second testing session ($\chi^2_1 = 2.20$, $P = 0.14$). Lines infected by Wolbachia were less active than uninfected lines ($\chi^2_1 = 4.99$, $P < 0.05$). Finally, the broad sense heritability of adult activity was 0.70 in males and 0.53 in females.

Correlation between adult activity and social behavior

Median nearest neighbor distances were not significantly correlated with measures of activity for either male or female flies (linear regression; $F_{1,27} = 2.92$, $P = 0.10$ and $F_{1,27} = 1.84$, $P = 0.19$, respectively; Figure 2c).

Correlations between larvae and adults

We found no significant correlation between measures of aggregation in larval and adult flies (linear regression; $r^2 = 0.05$, $F_{1,27} = 1.31$, $P = 0.26$; Figure 3a), nor was there any significant correlation in measures of activity between the 2 life stages (linear regression; $r^2 = 0.02$, $F_{1,27} = 0.54$, $P = 0.47$; Figure 3b). Finally, none of the correlations between larval and adult traits were significant when considering adult males and females separately (all $P > 0.27$). A power analysis (2 tailed, alpha = 0.05, beta = 0.20) revealed that our experimental design would allow one to reveal significant correlations for $r^2 > 0.24$ (Cohen 1988).

DISCUSSION

Our major findings were that there is a large genetic variation in both social behavior and activity in larval and adult fruit flies, that social behavior and activity are not positively correlated in either life stage, and that neither social behavior nor activity is coupled between larvae and adults. We discuss these findings in turn.

Genetic variation in social behavior

There has recently been increased interest in establishing simple model systems for research on the mechanisms and evolution of social behavior (Robinson et al. 2008; Sokolowski 2010). Our data, which indicate large genetic variation in social behavior in both larval and adult fruit flies (Figures 1a and 2a), further the establishment of fruit flies as a valuable model system in such research effort. Genetic variation in some aspects of social behavior has been documented in several species. In fruit flies, adult males from 5 distinct crosses of DGRP lines varied in their tendencies to join mixed-sex groups of different sizes (Saltz 2011). In the nematode *Caenorhabditis elegans*, naturally occurring morphs feed either alone or in groups. This difference in social behavior is determined by variation in the *npr-1* gene (de Bono and Bargmann 1998). In bees, the extensive work on the genetics of sociality has revealed a set of just over 200 genes that are shared by all the eusocial lineages studied (Woodard et al. 2011). In a variety of vertebrates, the neuropeptides oxytocin and arginine vasopressin modulate social behavior. Genetic variation in the *avpr1a* gene, which encodes one of the arginine vasopressin receptor subtypes, has been linked to variation in pair-bonding behavior in both voles and humans (Young et al. 1999; Donaldson and Young 2008; Walum et al. 2008; Ebstein et al. 2010). Although the work on the genetics of social behavior involves a variety of species (Hofmann et al. 2014), the establishment of robust protocols for quantifying social behavior in fruit flies can contribute to the overall research effort given the prevalence of tools available for fruit fly research across a multitude of disciplines from genetics and neurobiology to ecology and evolution.

Both larvae and adults of the DGRP genotypes that we tested showed a wide variation in their social tendencies as one would expect for a polygenic trait (Falconer and Mackay 1996). In the larvae, we found highly consistent variation in social scores between genotypes in distinct larval ages and in 2 experiments (Figure 1b). This is in spite of the variation caused by the dynamic nature of larval grouping (see Figure 1 in Durisko, Kemp, et al. 2014). Limited data we have for the adults also indicate high repeatability. In the adults, we found a highly significant interaction between sex and genotype (Figure 2a). In over half the lines we tested, the males were clearly less social than the females. This was expected given the antagonistic interactions among males in the context of their resource defense mating system (Hoffmann 1987; Chen et al. 2002; Saltz and Foley 2011; Baxter et al. 2015). Surprisingly, however, females were clearly less social than males in about one-third of the lines. This result deserves further mechanistic and functional investigations.

To quantify the degree of sociality between genotypes, we relied on the nearest neighbor index (Clark and Evans 1954). This index and similar measures have been instrumental in measuring social coherence in numerous enlightening studies of sociality in many species (White and Chapman 1994; Fischhoff et al. 2007; Evans and Harris 2008; Fero and Moore 2008; Grinblatt et al. 2008; Buijs et al. 2011). The main advantage of directly quantifying a major characteristic of the social group is that it reflects the outcome of social interactions among its members, which, in our study, were genetically identical. Hence, this group measure provides us with a comprehensive and objective measure for comparisons among genotypes and between life stages. Because an individual's social phenotype is determined by a variety of cues as well as interactions with other individuals, we are unlikely to have a satisfactory sociability score if we only focus on either a single cue or one sensory modality. Furthermore, such a narrow focus would prevent us from comparing between larvae and adults, which possess distinct sensory abilities and preferences. Nevertheless, it is clear that a complete characterization of social behavior in any species can benefit from a multitude of approaches.

The simplest explanation for the apparent grouping of individuals is individual attraction to a single specific site with preferred features such as temperature, moisture, shelter, food, or microbial composition. We critically tested and rejected this alternative in our previous work with larvae (Durisko, Kemp, et al. 2014), and our current protocol further reduces the possibility for such biases due to the switch from square to circular arenas, eliminating any irregularities caused by corners, as well as the replacement of our quadrat analysis with a continuous index. Furthermore, several controlled experiments indicated strong larval tendencies to join others, and larval learned preference to cues previously associated with others

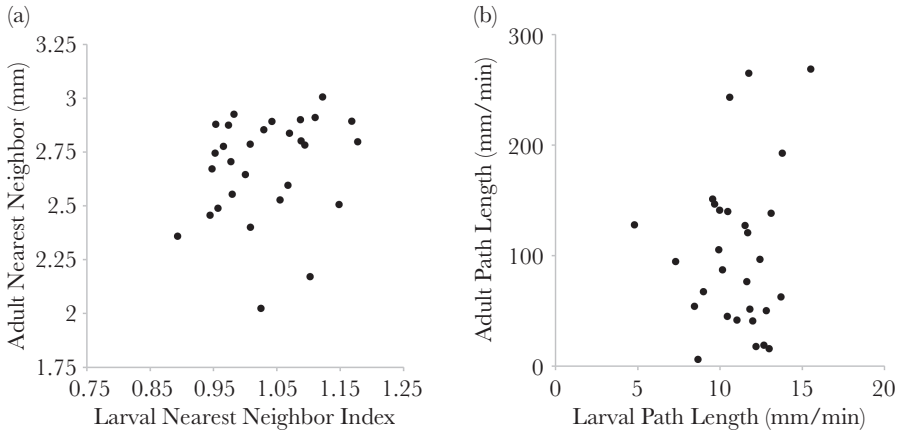

Figure 3
Relationship between larval and adult measures of (a) social behavior and (b) activity for the 29 DGRP lines tested. For adults, each point represents the average of the male and female scores.

(Durisko and Dukas 2013; Venu et al. 2014). Similarly, social attraction and interactions in the adults have been documented with distinct protocols in our (Sarin and Dukas 2009; Durisko, Anderson, et al. 2014) as well as a few other laboratories (Bartelt et al. 1985; Wertheim et al. 2006; Saltz 2011; Battesti et al. 2012; Schneider, Dickinson, et al. 2012; Simon et al. 2012).

Genetic variation in activity does not explain genetic variation in social behavior

Our major goal was to verify that our measure of social behavior was not merely an artifact of variation in levels of activity. For example, one could imagine that low activity individuals just remained at the center of the Petri dish where we initially placed them and thus appeared highly social, whereas highly active individuals dispersed randomly and hence looked nonsocial. Although we found large genetic variation in activity in both larvae and adults (Figures 1c and 2b), it was not positively associated with our measures of social behavior (Figures 1d and 2c). This finding substantiates our measure of social behavior as well as our use of the DGRP lines, which, although derived from wild flies, are inbred and thus could theoretically be inferior in their activity. We should note, however, that we tested only 72.5% of the lines we had initially received, and probably dropped all the weak lines. That is, even though we found no correlation between activity and social behavior, we think that activity should be measured along with the behavioral measure of interest in future studies because it may explain significant variation in traits such as mating success and aggression. Indeed such positive correlations between activity and other behaviors have been reported in other species. For example, in lake frogs, more active individuals were also more exploratory and bolder (Wilson and Krause 2012b). Similarly, in male water striders (*Aquarius remiges*), there was a positive association between activity, aggressiveness, and mating success (Sih et al. 2014).

In the adults, we found a highly significant interaction between sex and genotype (Figure 2b). Although males were clearly more active than females in over half the lines, females were more active than males in several genotypes. We expected males to be more active than females because they are the active sex when it comes to both resource defense polygyny (Emlen and Oring 1977; Baxter et al. 2015) and courtship. The intriguing information that females are more active than males in some genotypes deserves further research.

Finally, it is notable that our broad sense heritability estimates for social behavior (0.12 and 0.04 for larvae and adults, respectively) were much smaller than those for activity (0.42 and 0.62 for larvae and adults, respectively). The most likely explanation for this difference is that social behavior is the product of previous and current interactions among individuals. Hence, we would expect a strong environmental effect. In contrast, activity measured on isolated individuals can more strongly indicate consistent inherent individual propensities.

Larval and adult social behavior and activity are decoupled

Both social behavior and activity varied independently in larval and adult fruit flies (Figure 3). This outcome agrees with the adaptive decoupling hypothesis, which posits that antagonistic selection on larvae and adults has led to minimization of the genetic correlations between these life stages (Haldane 1932; Ebenman 1992; Moran 1994). By definition, metamorphosis implies an abrupt change in anatomy and morphology. This dramatic transformation indicates a large degree of decoupling between structural traits in larvae and adults. The case is less clear, however, for traits that are not easily observable such as physiology and behavior. As noted in the introduction, from an ultimate standpoint, one can readily invoke the adaptive coupling hypothesis, which states that the same characteristic may be equally beneficial in larvae and adults. Second, from a proximate perspective, the genetic architecture underlying certain traits may be too complex for allowing perfect decoupling. For example, adult male aggression is influenced by a large proportion of the genome and is affected by extensive epistasis and pleiotropy (Zwarts et al. 2011; Anholt and Mackay 2012; Shorter et al. 2015). This complexity might limit the degree of decoupling.

A growing body of literature indeed indicates imperfect decoupling between larvae and adults though it is not yet clear to what degree this reflects adaptation or constraint (Marshall and Morgan 2011). In the ascidian *Ciona intestinalis*, about 20% of the genes are similarly expressed in the larval and adult stages (Azumi et al. 2007). Furthermore, a genetic association between pre- and post-metamorphic viability has been documented in this species (Aguirre et al. 2014). Finally, at least 2 artificial selection experiments on

adult fruit flies had significant effects on larval traits. First, larval developmental rate was significantly lower in lines selected for late than for early-life fertility (Chippindale et al. 1994). Second, larval competitive ability was lower in lines selected for enhanced learning ability in the adults than in control lines (Mery and Kawecki 2003).

In conclusion, our results, which indicate decoupling of 2 central behavioral traits, social behavior and activity, between larvae and adults, agree with the adaptive decoupling hypothesis. Overall, though, the data from all studies to date detailed above and in the introduction suggest a mixture of coupling and decoupling of behavioral traits from larvae to adults. Theoretically, such a mix is actually predicted from both ultimate and proximate viewpoints. We thus suggest that future studies will be inclusive in addressing the 4 complementary hypotheses of adaptive decoupling as well as adaptive coupling, the genetic decoupling necessary for the development of 2 distinct life forms as well as the inherent genetic constraints underlying organismal complexity.

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