View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Serveur académique lausannois

ORIGINAL ARTICLE

doi:10.1111/evo.13115

Sexual selection shapes development and maturation rates in *Drosophila*

Brian Hollis,[1,2,3] Laurent Keller,[2] and Tadeusz J. Kawecki[2]

[1]*School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

[2]*Department of Ecology and Evolution, University of Lausanne, Biophore CH 1015 Lausanne, Switzerland*

[3]*E-mail: brian.hollis@epfl.ch*

Received September 8, 2016
Accepted November 2, 2016

Explanations for the evolution of delayed maturity usually invoke trade-offs mediated by growth, but processes of reproductive maturation continue long after growth has ceased. Here, we tested whether sexual selection shapes the rate of posteclosion maturation in the fruit fly *Drosophila melanogaster*. We found that populations maintained for more than 100 generations under a short generation time and polygamous mating system evolved faster posteclosion maturation and faster egg-to-adult development of males, when compared to populations kept under short generations and randomized monogamy that eliminated sexual selection. An independent assay demonstrated that more mature males have higher fitness under polygamy, but this advantage disappears under monogamy. In contrast, for females greater maturity was equally advantageous under polygamy and monogamy. Furthermore, monogamous populations evolved faster development and maturation of females relative to polygamous populations, with no detectable trade-offs with adult size or egg-to-adult survival. These results suggest that a major aspect of male maturation involves developing traits that increase success in sexual competition, whereas female maturation is not limited by investment in traits involved in mate choice or defense against male antagonism. Moreover, rates of juvenile development and adult maturation can readily evolve in opposite directions in the two sexes, possibly implicating polymorphisms with sexually antagonistic pleiotropy.

KEY WORDS: Development, *Drosophila melanogaster*, experimental evolution, maturation, sexual dimorphism, sexual selection.

Rates of juvenile development and maturation in animals often exhibit sexual dimorphism, leading to differences between males and females in age at maturity, a key life-history trait. However, our understanding of the evolutionary forces responsible for generating these sex differences is incomplete. Because postponed maturity implies additional risk of death before reproduction, and because there are often inherent advantages to shorter generation times, any delay in juvenile development or adult sexual maturation must be offset by gains to other components of fitness. In life-history theory these gains are usually assumed to be mediated by increased adult size conferring higher adult fitness (reviewed in Kozlowski (1992); Stearns (1992); Roff (1992)). This assumption has a broad empirical support in the case of females; in a wide range of taxa fecundity or offspring quality increase with female size (reviewed in Roff (1992); Honek (1993)).

For males, however, the reproductive advantages of large size are less general. In the absence of paternal care, male reproductive output is largely determined by success in competition for mating opportunities and sperm competition. Large size is an advantage to males in species where sexual selection mainly involves direct contests between males over breeding territories or access to females, such as many mammals and some birds, and this is thought to be an important factor promoting male-biased sexual size dimorphism (Hedrick and Temeles 1989). However, in most animal species, including almost all insects, males are smaller than females (Blanckenhorn et al. 2007), suggesting that gains in size derived from longer developmental periods may not be sufficiently beneficial to male sexual success to result in the evolution of longer duration male growth. In contrast, accelerated development may be favored when timing is important. For example, in species where generations are discrete and sexual selection consists primarily of scramble competitions for females that appear at a particular time of year and only mate soon after emergence, early maturity may be favored in males even if it comes at a

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

© 2016 The Author(s). *Evolution* © 2016 The Society for the Study of Evolution.

cost to adult size (Wiklund and Fagerström 1977; Fagerström and Wiklund 1982; Singer 1982; Zonneveld 1996). This is thought to have driven the evolution of faster male development (protandry) in butterflies (Singer 1982; Wiklund et al. 1991; Nylin et al. 1993), bees (Alcock 1997), and spiders (Maklakov et al. 2004). This early bird advantage does not apply in species where generations overlap, females are promiscuous, and sperm competition gains in importance. In such species males often take longer to develop from egg to adult than females but are still smaller as adults (Blanckenhorn et al. 2007), further suggesting that male reproductive success is less dependent on adult size than it is for females.

These arguments on the evolution of age at maturity neglect the fact that processes of maturation often continue after the animal has reached its final size (Roff 1992; Stearns 1992; Baker et al. 2003; Jones et al. 2007; Lemmen et al. 2016). It is not clear what selective forces and trade-offs shape the maturation process after final size is attained, and in particular whether sexual selection plays a major role. The time to reach reproductive maturity after growth has ceased could be chiefly determined by accumulation of resources for reproduction or maturation of the gametes (i.e., aspects of reproductive competence mostly independent of sexual selection). Alternatively, a major part of this maturation process in either sex could involve developing traits that mediate competition for mates, mate choice, sperm competition, and sexual conflict. This would grant sexual selection a major role in shaping age at maturity, a role that is independent of any age-size trade-offs. As an example of the latter scenario, sexual selection has been the driving force behind the evolution of unusually large and elaborate sperm in some *Drosophila* species (Lüpold et al. 2016), which in turn is thought to have required the evolution of prolonged posteclosion maturation in males (Pitnick et al. 1995). It remains an open question how general the role of sexual selection is in shaping maturation after final size has been reached in males and females of species that are less sperm limited.

To address this question, we investigated the role of sexual selection in determining age at maturity of male and female *Drosophila melanogaster* using long-term experimental populations that have been evolving in manipulated mating systems (Hollis and Houle 2011). Three populations have evolved for over 100 generations without sexual selection, achieved by imposing randomized monogamy that eliminated pre- and postcopulatory competition between males as well as mate choice. In parallel, three populations of the same origin were maintained under a controlled polygamous regime and continued to experience sexual selection. Under both regimes, flies were only allowed to eclose, mate, and oviposit within short-time windows, which imposed selection for fast development and maturation. Flies that took too long to eclose would not be included in the mating pool, and those taking too long to mature would not fully realize their reproductive potential in the time window available. The key question we asked is whether the presence versus absence of sexual selection altered the strength or form of total selection on age at maturity, leading to the evolution of differences between the monogamous and polygamous populations in sex-specific preadult developmental time or posteclosion maturation rates. We focused on these two traits because they jointly determined how mature flies were during the mating and reproduction time window in the experimental evolution regimes. If the process of maturation were mostly about developing the capacity to mate and produce viable sperm in sufficient quantity (in males) or achieve maximum fecundity (in females)—aspects of reproduction independent of sexual selection—then developmental time and maturation rate would not be expected to evolve differently in the monogamous and polygamous populations. In contrast, if an important part of male maturation involved gearing up for sexual competition, removal of sexual selection would reduce the advantages of early maturity and lead to the evolution of longer development and/or slower maturation of males, particularly in light of the known costs to viability that accompany accelerated development (Chippindale et al. 1997; Prasad et al. 2000). Similarly, if an important part of maturation in females involved preparing physiologically or cognitively for antagonism from males and mate choice, one would expect this aspect of selection to be relaxed under monogamy. This would yield a similar prediction of slowed female development and/or maturation evolving under monogamy. However, because developmental time of the sexes is known to be positively genetically correlated in *Drosophila* (Chippindale et al. 1997) and other insects (Zwaan et al. 2008), and the same is likely for posteclosion maturation rate, any difference in selection on those traits in one sex might lead to parallel changes in both.

To test these predictions we used two complementary approaches. First, we compared developmental time and the rate of maturation of males and females from the evolved monogamous and polygamous populations. Developmental time was defined as the period from egg to eclosion of the adult from the pupal case (at which point it is not yet sexually mature). Because posteclosion maturation is difficult to assess at the level of visible phenotypes, we compared the rate of maturation of flies from the monogamous and polygamous populations with a novel approach based on the maturation trajectory of the transcriptome. We initially determined which genes change in expression with age using an independent sample of *D. melanogaster*. Based on the pattern of change in these genes, we then assessed the degree of maturity of 4-day old male and female flies from all six of the evolved populations.

Second, in an independent experiment we investigated direct phenotypic selection on age at maturity under both monogamous and polygamous regimes. We assessed the fitness consequences

of being more or less mature by quantifying the competitive reproductive success of 3-, 4-, and 5-day old individuals from the ancestral population when confronted with standardized mates and competitors.

Consistent with a role of sexual selection in shaping male maturation, males from evolved monogamous populations took longer to develop and were transcriptionally less mature 4 days after eclosion than males from the polygamous populations. This corresponded to the results of the phenotypic selection assay, which indicated that only the polygamous regime selects for fast male maturation. However, the corresponding results for females contradicted the predictions: monogamous females developed faster and showed a signature of greater transcriptomic maturity at 4 days of eclosion than females from the polygamous populations, in spite of phenotypic selection for early female maturity appearing equivalently strong under both regimes.

In an attempt to explain these results we tested for changes in two fitness components that are commonly involved in trade-offs with age at maturity: adult size (Hillesheim and Stearns 1991, 1992) and survival to adulthood (Chippindale et al. 1994; Prasad et al. 2000). First, given that longer development allows more time to grow, we considered the possibility that these differences could have evolved as correlated responses to sexual selection on body size in either sex. If this were the case, the monogamous populations should have evolved a larger male size and a smaller female size compared to the polygamous populations. We tested this prediction by measuring adult weight of individuals of both sexes emerging across a range of developmental times. Second, we considered the possibility of a sex-specific trade-off between early maturity and high juvenile mortality rate. If such a trade-off contributed to the evolution of the fast female and slow male development under monogamy, the monogamous populations should have evolved a lower female but higher male egg-to-adult survival compared to the polygamous populations.

Materials and Methods

FLY POPULATIONS, REARING, AND EXPERIMENTAL EVOLUTION DESIGN

Experiments were carried out with several populations of *D. melanogaster*, all derived originally from a long-term laboratory-adapted population designated IV (Charlesworth and Charlesworth 1985). Our base IV population has been maintained at several thousand individuals, across 10 bottles, with flies mixed and moved to new media on a 14-day schedule. Because the IV population has been maintained at high density, there is strong selection for fast development (Houle and Rowe 2003).

To study the consequences of sexual selection, six experimentally evolving populations were established from the IV population in 2007 after a mutagenesis treatment that elevated levels of standing genetic variation for fitness and maintained, at a census size of 200 adults, under either monogamous or polygamous regimes (Hollis and Houle 2011). In the three monogamous populations, virgin females are randomly paired with virgin males and spend two days together in interaction vials. In the polygamous populations, groups of five virgin females are combined with five virgin males and also spend two days together in interaction vials. After this two day period, males from all populations are discarded and females are placed into two bottles per population, with 50 females in each bottle. The mated females then spend three days laying eggs in these bottles before also being discarded. Offspring are collected in the first days of emergence as virgins (normally 11 and 12 days after egg laying in the preceding generation commenced) and passed back through the selection treatment. Thus, flies under the two regimes experience the same developmental conditions and the same oviposition environment and only differ in the number of competitors and potential mates during the 2-day mating period.

The measures of adult maturation, egg-to-adult development time, and adult dry mass described below were always preceded by one generation of rearing under standardized conditions to control for nongenetic effects of the maternal mating environment. All flies were reared on 2% yeast media (water, agar [Milian CH], brewer's yeast [Migros CH], cornmeal, sucrose, and Nipagin [Sigma-Aldrich CH]) and maintained on a 12L:12D photoperiod at 25°C

EGG-TO-ADULT DEVELOPMENT TIME

We measured egg-to-adult development time in our six evolved populations after 139 generations of experimental evolution. We did this in a competitive setting, using a standardized ebony competitor from a population that originates from and is maintained in the same manner as the IV population. The recessive ebony phenotype of dark body coloration allows these flies to be easily distinguished from those with wild-type body coloration.

We placed five males and five females from a given population together for two days in vials, then moved each set of females to a bottle with 45 inseminated ebony competitor females ($n = 4$ bottles/population). Males were discarded. After three days of egg-laying, all females were discarded. Male and female offspring were counted daily as they eclosed, giving us sex-specific measures of development time for all populations. Using a standardized competitor allowed us to match the density of both females during egg-laying and larvae during development as closely as possible to the selection regimes, while at the same time limiting within-population competition. We compared average developmental time (weighted by the number of individuals eclosing on each day post-egg laying) with a linear-mixed model in SAS 9.2 (SAS Institute 2011) PROC GLIMMIX. The model

included selection regime and sex, along with the interaction, as fixed effects, and replicate population nested within selection regime as a random effect. We also included experimental bottle as a random effect, as many flies eclosing from each bottle were scored. We also examined the sex ratio of the emerging flies to determine whether there were differences in sex-specific viability between the regimes (direct quantification of sex-specific viability is not possible because eggs or newly hatched larvae are impractical to sex). We analyzed this with a generalized linear-mixed model in PROC GLIMMIX with the number of males out of the total number of emerged flies as the response variable and the same set of fixed and random effects as in the developmental time model.

TRANSCRIPTOMIC MATURITY

Quantifying maturity is challenging at the level of visible phenotypes, particularly without a priori knowledge of the relevance of the phenotypes to sexual success and fitness. We therefore assessed the rate of sexual maturation of male and female flies from our monogamous and polygamous populations using whole-transcriptome gene expression profiles. Specifically, we scored gene expression of our flies at 4 days of age on a transcriptomic maturity axis obtained from an independent dataset (the modENCODE project (Celniker et al. 2009)). This was done using gene expression in fly heads rather than whole bodies, which avoids confounding effects of potential differences in gonad size between the monogamous and polygamous populations.

Whole-transcriptome gene expression profiles from the adult heads of flies from our monogamous and polygamous populations were collected after 117 generations of experimental evolution as part of a previous study focused on sex-biased gene expression (Hollis et al. 2014). Briefly, all six evolved populations were reared in the monogamous mating system for one generation. Next, the heads of 4-day old males and females were dissected into liquid nitrogen (~100 heads/sex/replicate population). This was followed by RNA extraction, cDNA library generation, and sequencing with an Illumina HiSeq 2500 (four lanes, all 12 libraries multiplexed on all lanes, single end chemistry). Reads were mapped to the *D. melanogaster* transcriptome using Tophat 2 (Kim et al. 2013) and assigned to features (genes) using HTSeq (http://www–huber.embl.de/users/anders/HTSeq/). Final coverage was between 34 and 53 million reads per sample.

To define an axis of maturity, we used independent gene expression data from the modENCODE project (Celniker et al. 2009) that comes from 1-day and 4-day old male and female heads of the Oregon-R strain (two biological replicates for each age by sex combination). These data were obtained from the Gene Expression Omnibus and reads were mapped and assigned to features in the same manner as for the evolved populations. Final coverage was between 25 and 82 million reads per sample.

Count data for all samples were next normalized by total library size in the DESeq2 package (Anders and Huber 2010) of the Bioconductor suite (Gentleman et al. 2004). The 40% of genes with the lowest expression levels in males (for the male analysis) and females (for the female analysis) were filtered out, leaving 9408 genes for downstream analysis. We then fit linear models on the modENCODE counts for these genes, with a single effect of age, for each sex separately. From these tests, we generated a list of the 50 genes with the lowest Benjamini–Hochberg adjusted *P* values for each sex as markers for transcriptomic maturity.

Assuming linear change in expression from 1 to 4 days old for each gene in this list, we calculated a transcriptomic maturity score (M, in days) for males and females from our six evolved populations separately for each gene as:

$$M_P = \frac{(\text{expression}_p - \text{expression}_{\text{age1}})}{(\text{expression}_{\text{age4}} - \text{expression}_{\text{age1}})} \times 3 + 1$$

where p is an evolved population, age1 is the Oregon-R 1-day old individuals from the modENCODE data, and age4 is the Oregon-R 4-day old individuals from the modENCODE data. Because estimates of maturity vary greatly from gene to gene, we calculated a residual maturity by subtracting the mean maturity across all six populations from our maturity estimates for each population, for each gene. We modeled residual maturity using a linear-mixed model with selection regime as a fixed effect and replicate population nested within selection regime as a random effect. Note that with this approach, we are not able to compare the maturity scores of our fly populations to those used in the modENCODE project, due to differences in experimental protocols and genetic background as well as statistical biases that might be introduced by the use of the modENCODE flies to calibrate our maturity measures. However, the transcriptomic maturity scores can be fairly compared between our own populations and selection regimes, for which these aspects are controlled. Another caveat with this approach is that, because we are looking at gene expression in only the head, any differences we detect can in principle be restricted to the head and therefore not be indicative of the differences present in other parts of the fly relevant to sexual reproduction (e.g., the male and female reproductive tissues).

PHENOTYPIC SELECTION ON MATURITY

To assess the fitness consequences of being more or less mature we quantified the competitive reproductive success of 3-, 4-, and 5-day old individuals confronted with 4-day old mates and competitors. The relatively young or old flies served as a proxy for genetic variation conferring slower or faster maturation, respectively. This assay was done under conditions mimicking the monogamous and polygamous regimes, using flies from the base IV population from which the monogamous and polygamous populations were originally derived.

To collect flies for use in the assays that were consistently some of the first to eclose from their bottles, while simultaneously allowing all subsequent assays to be established on the same day, we used the following scheme. We first established multiple bottles, each with approximately 100 adults from the IV population. The next day, a second set of bottles was established by transferring the same adults. This was repeated again on the third day, and one day later all adult flies were discarded. In this way, we established replicate bottles staggered across three days. We then collected some of the first emerging male and female flies from these bottles as virgins. Those flies that would be aged to 5 days old were collected from the first set of established bottles. One day later, flies that would be aged to 4 days old were collected from the second set established bottles. One day later, flies that would be aged to 3 days old were collected from the third set of established bottles. The collected virgins were housed individually and aged to either 3, 4, or 5 days before the assays began.

To measure competitive reproductive success in the polygamous regime, we placed individuals of each sex and each age class in competition with four 4-day old ebony individuals of the same sex, and five 4-day old ebony individuals of the opposite sex. These flies were left for two days, at which point the five females in each vial were moved to a new vial and the males discarded. Females were then allowed to lay eggs for three days before being discarded. For measures in the monogamous regime, we placed individuals of each sex and each age class with one 4-day old ebony individual of the opposite sex. For each vial containing one focal individual, we set up four corresponding vials with one 4-day old ebony male and one 4-day old ebony female. As in the polygamous treatment, all flies were left for two days, at which point five females, one of whom was the focal individual and four who were ebony, were moved to a new vial and the males discarded. Females were then allowed to lay eggs for three days before being discarded.

From all resulting vials, we collected emerging offspring and scored body coloration to determine whether they were the progeny of the focal individual. Because all competitor flies in each replicate were ebony, all wild-type progeny belonged to the focal individual. The entire experiment was run twice, yielding two experimental blocks.

We analyzed the proportion of individuals that were wild type in appearance out of the total number of offspring (competitive fitness) with generalized linear-mixed models in SAS 9.2 (SAS Institute 2011) PROC GLIMMIX. For each sex, we used a separate GLMM with mating system and age as fixed effects, along with the mating system by age interaction. We included experimental block as a random effect. Because our primary interest was in the difference between the two mating systems in the change in reproductive success across age classes (the mating system by age interaction), for visualization we normalized each sex and mating system combination by mean fitness.

DRY MASS

We measured dry mass of males and females eclosing from the evolved populations after 162 generations of experimental evolution. We placed groups of five virgin males and five virgin females together for two days, for each of the six populations. We then discarded all males and placed females in groups of 50 (two bottles/population) and allowed the females to lay eggs for three days. We then collected and froze adults on the day they emerged across 10, 11, or 12 days of development time. We later dried these flies for 12 hours at 60C and weighed them individually using a microbalance ($n = 5$ individuals/sex/day of eclosion/population, for 180 total measures). We then fit a generalized linear-mixed model for each sex in SAS 9.2 (SAS Institute 2011) PROC GLIMMIX with dry mass as the response variable and selection regime and day of eclosion as fixed effects, along with the interaction. We included population as a random effect nested within selection regime.

Results

EGG-TO-ADULT DEVELOPMENT TIME

Selection regimes had contrasting effects on the egg-to-adult development time of the two sexes (regime × sex interaction: $F_{1,22} = 22.00$, $P < 0.001$). While males from monogamous populations took more time to develop to the adult stage than males from polygamous populations, by an average of 4.2 hours (pairwise contrast, $t_{22} = 2.91$, $P = 0.008$, Fig. 1A and B), females from monogamous populations developed on average 3.6 hours faster than females from polygamous populations ($t_{22} = 2.50$, $P = .020$, Fig. 1C and D). This also means that the magnitude of sexual dimorphism in development time differed between regimes; while monogamous females developed on average 7.6 hours faster than males (pairwise contrast, $t_{22} = 6.39$, $P < 0.0001$), in the polygamous regime the difference between female and male development was minimal (pairwise contrast, $t_{22} = .24$, $P = 0.809$).

TRANSCRIPTOMIC MATURITY

We calculated a measure of transcriptomic maturity based on the top 50 gene expression markers for age, derived independently for males and females, for all of the evolved populations. Despite measuring expression profiles for flies that all shared the exact same chronological age of 4 days posteclosion, we found significant differences in the maturity of populations that had evolved in different selection regimes. Males from all three evolved monogamous populations were transcriptionally younger than males from all three polygamous populations when examining the median “transcriptional age“ estimates across all marker

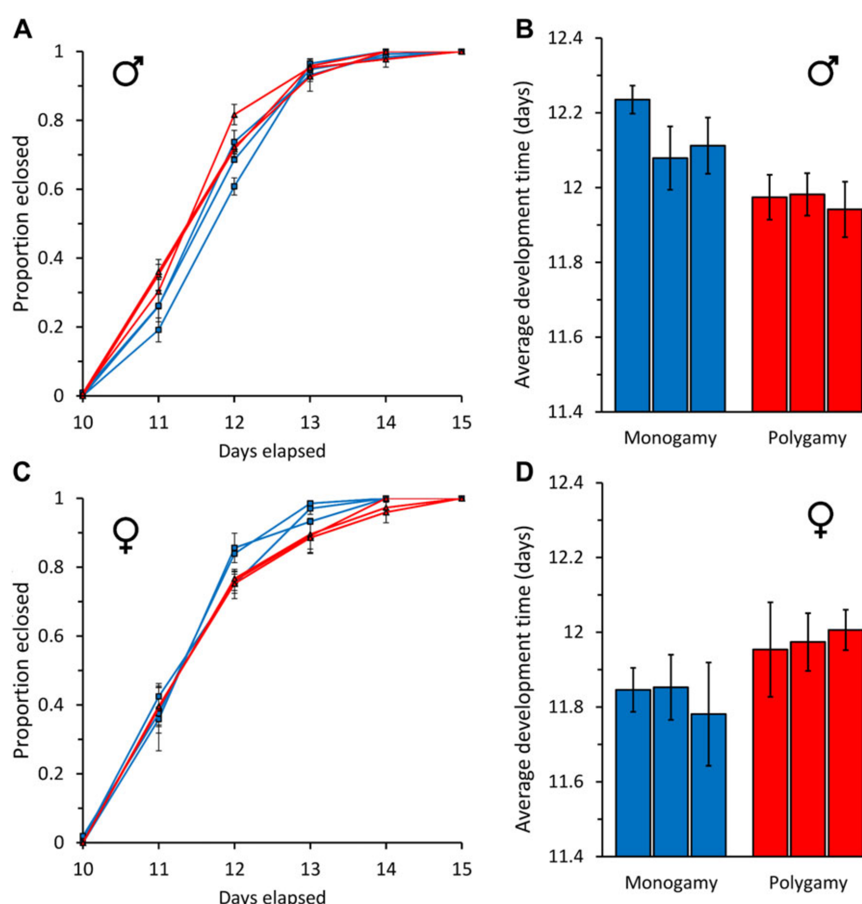

Figure 1. Male (A and B) and female (C and D) egg-to-adult development across the six evolved populations. The proportion of all adults (± S.E.) that had eclosed by each of six days post egg-laying is shown in panels A and C, and the weighted average egg-to-adult developmental time (± S.E.) derived from these curves is shown in panels B and D. Monogamous populations are depicted in blue and polygamous populations in red.

genes (3.78, 3.78, and 3.74 days for the three monogamous populations, vs 3.94, 3.87, and 3.89 for the three polygamous populations, Supporting Information S1). We tested for an effect of selection regime by modeling a standardized maturity score (the gene-specific age estimate for a population minus the mean age estimate for that gene across all populations). This difference in male transcriptomic maturity between selection regimes was significant ($F_{1,4} = 32.5$, $P = 0.005$, Fig. 2A). On average, males from monogamous populations had transcriptomes that were 3.3 hours less mature. This effect is evident across the breadth of the transcriptome—of the marker genes derived from the modENCODE male data, 43 out of 50 (86%) showed a less mature expression profile on average in the monogamous regime relative to the polygamous regime.

In females, we found an effect in the opposite direction. Monogamous females from all three evolved monogamous populations appeared older transcriptionally than females from all three polygamous populations when evaluating median age estimates across all genes (3.98, 3.94, and 3.95 for the three monogamous populations, vs 3.93, 3.83, and 3.83 for the three polygamous populations, Supporting Information S2). The overall difference between selection regimes was significant in the model of standardized maturity scores that accounted for gene-to-gene noise ($F_{1,4} = 8.9$, $P = 0.040$, Fig. 2B). On average, females from monogamous populations had transcriptomes that were 2.3 hours more mature than their polygamous counterparts. Of the marker genes for age from the modENCODE female data, 48 out of 50 (96%) show a more mature expression profile on average in the monogamous regime relative to the polygamous regime.

PHENOTYPIC SELECTION ON MATURITY

Differences in developmental time and posteclosion maturation rate reported above might have evolved because the removal of sexual selection changed the fitness consequences of being more or less mature. To test this hypothesis, we studied the reproductive fitness of 3-, 4-, or 5-day old individuals from the ancestral population when confronted with 4-day old competitors and mates, under the conditions corresponding to either the monogamous or the

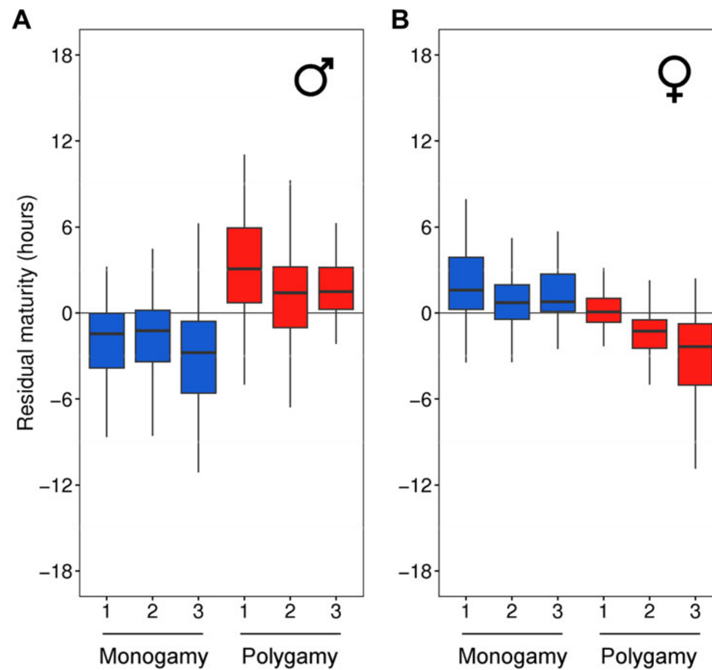

Figure 2. Residual maturity scores for males (A) and females (B) from the six evolved populations, in hours. The 50 genes that show the strongest evidence for change in expression between 1 and 4 days of age in the modENCODE dataset, determined separately for each sex, are included as markers of maturity. For each gene, residual maturity is calculated as the difference of a given population's maturity score from the mean of all six populations. Whiskers extend to 1.5x the interquartile range.

polygamous regime. The mating regime strongly affected the relationship between male age and fitness (age × regime interaction, $F_{1,129} = 8.42$, $P = 0.004$, Fig. 3A, B). Age did not detectably affect the focal male's fitness under the monogamous regime ($t_{129} = 0.99$, $P = 0.325$, Fig. 3A). In contrast, under the polygamous regime, with sexual selection operating, male fitness increased with age; 5-day old males had a 28% greater offspring share than 3-day old males ($t_{129} = 3.43$, $P < 0.001$, Fig. 3B).

In contrast to the male results, the mating regime did not affect the relationship between female age and fitness (age × mating system interaction, $F_{1,127} = 0.01$, $P = 0.940$, Fig. 3C, D). Older females had higher competitive reproductive success than younger ones under both monogamous ($t_{127} = 4.17$, $P < 0.001$, Fig. 3C) and polygamous mating regimes ($t_{127} = 4.02$, $P < 0.001$, Fig. 3D), with 5-day old females in both settings having 44% higher offspring share than 3-day old females.

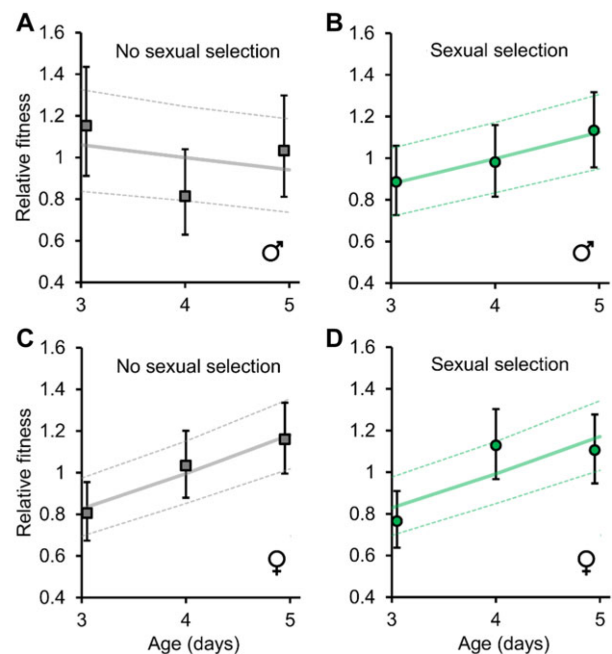

Figure 3. Relative fitness (± S.E.) of focal males (A and B) and females (C and D) of three different ages (3, 4, or 5 days old) when placed in either a monogamous or polygamous regime with 4-day old *ebony* male and female competitors. Fitness is mean-standardized within each sex × regime combination. The solid and dashed lines illustrate model predictions and error bands (± S.E.), respectively.

DRY MASS

We found no significant effect of selection regime ($F_{1,4} = 0.00$, $P = 0.979$), day of emergence ($F_{1,82} = 0.12$, $P = 0.728$), or the interaction ($F_{1,82} = 0.01$, $P = 0.908$) on male body weight (Fig. 4A). Likewise, there was no effect of selection regime ($F_{1,4} = 2.58$, $P = 0.183$) or the selection regime x day interaction on female dry mass ($F_{1,82} = 2.31$, $P = 0.133$), although day of emergence mattered for body weight in females ($F_{1,82} = 82.19$,

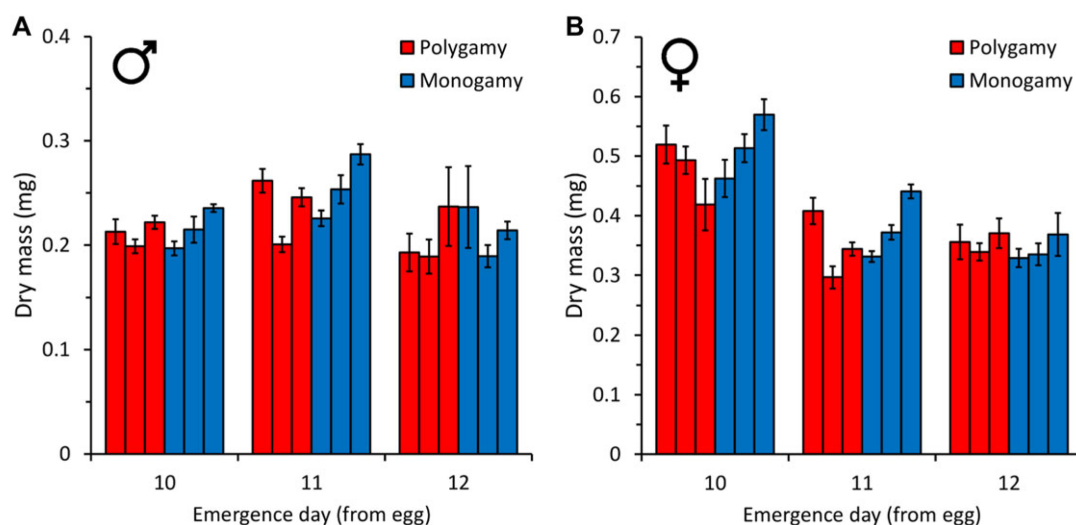

Figure 4. Dry mass (± S.E.) of males (A) and females (B) from each of the six evolved populations, across the first three days of emergence.

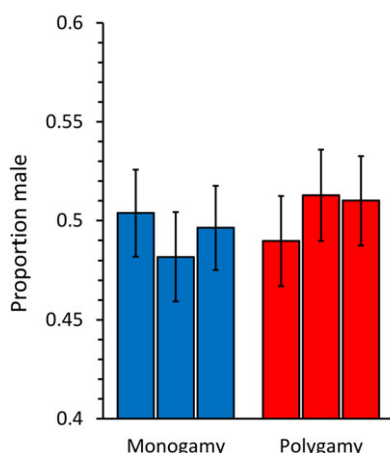

Figure 5. The proportion of all emerged flies (± S.E.) from the egg-to-adult development time assay that was male, from each of the six evolved populations.

$P < 0.001$, Fig. 4B)—females emerging on the last day measured (day 12) had on average 30% lower dry mass than those emerging on the earliest day (day 10).

RELATIVE VIABILITY OF THE SEXES

We analyzed the sex ratio of emerging flies from our egg-to-adult development time experiment to assess whether there were differences between the regimes in sex-specific viabilities. We found no difference between monogamous and polygamous regimes in the proportion of males out of the total offspring ($F_{1,4} = 0.29$, $P = 0.617$, Fig. 5). On average in each regime, 49.4% of monogamous (95% CI 45.9–52.9%) and 50.4% of polygamous (95% CI 46.8–54.1%) offspring were male, suggesting no evolved differences in relative viability of the sexes.

Discussion

The aim of our study was to test for the role of sexual selection in shaping posteclosion maturation of males and females in *D. melanogaster*. We hypothesized that an important aspect of this process may be preparing the individual for competition for mates, mate choice, sexual antagonism, and sperm competition. If this were the case, elimination of sexual selection by randomized monogamy would relax selection on fast maturation, despite the short-generation cycle imposed on the experimental populations, leading to the evolution of slower posteclosion maturation and/or longer developmental time. Furthermore, as an independent test of the role of sexual selection in shaping maturation rate, the advantage of being older in our phenotypic fitness assay should have been greater under the polygamous than the monogamous regime.

These predictions were supported for males. Males from populations evolved under the monogamous regime had slower egg-to-adult development times and transcriptomes that appeared several hours younger than age-matched polygamous males. These findings are in line with the phenotypic fitness assay that showed a clear advantage for older males under the polygamous regime, but no such advantage under the monogamous regime. These results demonstrate that important aspects of the maturation process contribute to male success in sexual competition. Such success could be mediated either through development of sexual signals (e.g., cuticular hydrocarbons, which continue to change for several days after eclosion (Arienti et al. 2010), or motor and cognitive abilities involved in courtship (Hollis and Kawecki 2014)), or through development of physiological traits involved in postcopulatory sexual selection, like sperm and seminal fluid production. In line with this idea, there is evidence that sperm number increases in the first days after eclosion (Pitnick et al. 1995) and the size of the male accessory glands, where nearly all of the

seminal fluid proteins are produced, is increasing for at least the first 6 days after eclosion (Ruhmann et al. 2016). Investment by males in traits like these that are responsible for improving sexual competiveness would not be favored in the absence of sexual selection, with the caveat that some of the seminal fluid proteins aid in sperm storage and boost female fecundity and would therefore still have value for males in the absence of male–male competition.

In contrast to the evolutionary change observed in males, evolved females showed faster egg-to-adult development and posteclosion maturation rate under monogamy than under the polygamous regime. However, the assay of the relationship between female age and fitness indicates that this is not because the polygamous regime favored females that were less mature. On the contrary, under both regimes 5-day old females had about 40% higher fitness than 3-day old females, implying that both regimes strongly and similarly favored females that were more mature during the reproductive time window, likely because the maturation process involves an increase in fecundity (McMillan et al. 1970). Thus, the evolved differences between monogamous and polygamous populations in female development and maturation rate are unlikely to have been driven by the contribution of sexual selection or conflict to direct selection on the rate of maturation.

An alternative potential explanation for the faster development and maturation in females under the monogamous regime is that it is a correlated response to a difference between the regimes in selection on some other trait or traits. In particular, if the monogamous regime relaxed selection on a fitness-relevant female trait that traded off genetically with early maturation, the populations should evolve toward early maturation at the expense of that other trait, even if direct selection on maturation remained unchanged. Correlated responses to selection on other traits might have also contributed to the evolution of slower male development and maturation under monogamy. Even though our data indicate no advantage for males of being more mature under monogamy, they do not support an advantage of being less mature. This implies that delayed male maturation was not favored under monogamy because it, for example, reduces male harm to the female, as this effect would also operate in the phenotypic selection assay. Therefore, the delayed development and maturation of males is unlikely to be a response to direct selection against early maturation. Rather, it could have been driven by a trade-off with another fitness-related trait that remained under selection under monogamy (e.g., viability), and which was thus freer to evolve once selection on male maturation was relaxed through the monogamy regime. If this explanation were correct, the faster female development under monogamy should have been accompanied by a reduction in some other fitness-related trait in females, whereas the slower male development of monogamous populations should have been compensated by an improvement of another male fitness component. To assess this possibility, we assayed two traits known to trade-off with the rate of development in *Drosophila* and other insects: adult body size and egg-to-adult viability (Chippindale et al. 1997; Nylin and Gotthard 1998; Prasad et al. 2000). The adult weight of either sex did not differ between the selection regimes, regardless of individuals' egg-to-adult development time, nor did the male/female ratio at eclosion (indicative of the relative male vs female survival to adulthood). We therefore found no evidence that faster female development in the monogamous populations traded off with egg-to-adult survival or adult body size of females, or that the slower development of monogamous males was compensated for by better survival or larger size. Thus, the trade-off scenarios laid out above are not supported by the body size or egg-to-adult viability data, although trade-offs involving some other fitness components like investment in defense against male harm cannot be excluded.

One final potential explanation for our results is that the divergence between the monogamous and polygamous populations has been mediated by alleles with antagonistic effects on the age at maturity in the sexes. Under polygamy, this scenario would predict an equilibrium in which the marginal fitness gain for females from earlier maturity would be equalized by marginal fitness loss for males from delayed maturity and vice versa. Because the monogamous regime relaxes selection on early maturity in males, this equilibrium trade-off would be expected to shift in favor of females, explaining the evolution of both fast females and slow males. This hypothesis would also explain the apparent absence of costs to earlier maturity in monogamous females—the costs would be borne by males. The main problem with this sexually antagonistic pleiotropy hypothesis is that r_{mf}, the intersexual genetic correlation, is high and often close to 1 for most traits (Roff and Fairbairn 1993; Poissant et al. 2010), including egg-to-adult developmental time in *Drosophila* and other insects (Chippindale et al. 1997; Prasad et al. 2000; Zwaan et al. 2008). Because of this high r_{mf}, two different male-limited experimental evolution studies have shown males and females evolving in the same direction—becoming more masculine—for several phenotypes including development time, body size, and wing shape (Prasad et al. 2007; Abbott et al. 2010). Thus the developmental time of the two sexes evolving in opposite directions in the absence of sexual selection is rather unexpected.

On the other hand, r_{mf} is a summary parameter and polymorphisms with sexually antagonistic effects are likely to be present despite a highly positive r_{mf}. Even if loci with sexually antagonistic effects in general contribute a minor part of genetic variation in the rates of development and maturation, they might have contributed disproportionally to the divergence between the polygamous and monogamous populations. The base population had been maintained under a short-generation time, intense

sexual selection, and high competition for food (Houle and Rowe 2003) for over 700 generations before it was used to establish the experimental populations. Alleles that accelerate development of one or both sexes without substantial trade-offs should have been driven to high frequency or fixed. In contrast, theory predicts sexually antagonistic pleiotropy for a trait under directional selection to be a powerful mechanism maintaining polymorphism (Levene 1953; Rice 1984). Allele frequencies at such polymorphic loci would be expected to respond rapidly to a change in the balance of selection on the two sexes. Consistent with this, by applying artificial selection for fast male and slow female development and vice versa, Zwaan et al. (2008) succeeded in changing the degree of sexual dimorphism in developmental time in a butterfly, despite a strongly positive r_{mf}. Sexually antagonistic pleiotropy is therefore a viable hypothetical explanation for the contrasting effects of the removal of sexual selection on the evolution of male and female development and maturation rate that can be explored further by studying the genetic architecture of these traits.

Irrespective of the genetic architecture underlying the evolutionary changes we report, our results lead to two conclusions. First, the rate of maturation of the two sexes can evolve in opposite directions rapidly enough to be observed in the lifetime of an experimental evolution study. This can lead to evolutionary changes in sexual dimorphism: whereas in the monogamous populations females eclosed from pupae on average almost 8 hours earlier than males, in the polygamous populations this difference virtually disappeared.

Second, sexual selection is an important force shaping the posteclosion maturation processes of male *D. melanogaster*. We have demonstrated this under typical laboratory culture conditions characterized by discrete generations with a short-generation time. However, we believe that our results are also relevant for understanding the evolution of age at maturity in nature, although not through a simple extrapolation. A key factor in sexual selection on early male maturation in our polygamous regime was the limitation of mating opportunities to a short time window early in adult life. This factor is likely less severe under natural conditions, where *Drosophila* generations are overlapping and mating opportunities occur throughout a male's life. Therefore, our results do not imply that sexual selection under natural conditions favors fast maturing males generally. Rather, they show that sexual selection is a major factor in determining the time it takes to reach full maturity, and whether this leads to relatively fast or slow males will depend on the details of the mating system that ultimately decide how male sexual success is achieved.

ACKNOWLEDGMENTS

We thank E. Genzoni for help with experiments and K. Harshman and the Lausanne Genomic Technologies Facility for sequencing support. Computations were performed at the Vital-IT Center for high-performance computing (http://www.vital–it.ch) of the SIB Swiss Institute of Bioinformatics. This work was supported by Swiss National Science Foundation grants to T.J.K. and L.K., as well as an ERC Advanced grant to L.K.

DATA ARCHIVING

All phenotypic data are available from the Dryad Digital Repository at http://dx.doi.org/10.5061/dryad.2973n. RNA-Seq data have been deposited at the GEO under the accession code GSE50915.

LITERATURE CITED

Abbott, J. K., S. Bedhomme, and A. K. Chippindale. 2010. Sexual conflict in wing size and shape in *Drosophila melanogaster*. J. Evol. Biol. 23:1989–1997.

Alcock, J. 1997. Small males emerge earlier than large males in Dawson's burrowing bee (*Amegilla dawsoni*) (Hymenoptera: Anthophorini). J. Zool. 242:453–462.

Anders, S., and W. Huber. 2010. Differential expression analysis for sequence count data. Genome Biol. 11:R106.

Arienti, M., C. Antony, C. Wicker-Thomas, J. P. Delbecque, and J. M. Jallon. 2010. Ontogeny of *Drosophila melanogaster* female sex-appeal and cuticular hydrocarbons. Integr. Zool. 5:272–282.

Baker, R. H., M. Denniff, P. Futerman, K. Fowler, A. Pomiankowski, and T. Chapman. 2003. Accessory gland size influences time to sexual maturity and mating frequency in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. Behav. Ecol. 14:607–611.

Blanckenhorn, W. U., A. F. G. Dixon, D. J. Fairbairn, M. W. Foellmer, P. Gibert, K. van der Linde, R. Meier, S. Nylin, S. Pitnick, C. Schoff, et al. 2007. Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? Am. Nat. 169:245–257.

Celniker, S. E., L. A. L. Dillon, M. B. Gerstein, K. C. Gunsalus, S. Henikoff, G. H. Karpen, M. Kellis, E. C. Lai, J. D. Lieb, D. M. MacAlpine, et al. 2009. Unlocking the secrets of the genome. Nature 459:927–930.

Charlesworth, B., and D. Charlesworth. 1985. Genetic-variation in recombination in Drosophila.1. Responses to selection and preliminary genetic-analysis. Heredity 54:71–83.

Chippindale, A. K., J. A. Alipaz, H. W. Chen, and M. R. Rose. 1997. Experimental evolution of accelerated development in Drosophila.1. Developmental speed and larval survival. Evolution 51:1536–1551.

Chippindale, A. K., D. T. Hoang, P. M. Service, and M. R. Rose. 1994. The evolution of development in *Drosophila melanogaster* selected for postponed senescence. Evolution 48:1880–1899.

Fagerström, T., and C. Wiklund. 1982. Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. Oecologia 52:164–166.

Gentleman, R. C., V. J. Carey, D. M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. C. Ge, J. Gentry, et al. 2004. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5:R80.

Hedrick, A. V., and E. J. Temeles. 1989. The evolution of sexual dimorphism in animals: hypotheses and tests. Trends Ecol. Evol. 4:136–138.

Hillesheim, E., and S. C. Stearns. 1991. The responses of *Drosophila melanogaster* to artificial selection on body-weight and its phenotypic plasticity in 2 larval food environments. Evolution 45:1909–1923.

———. 1992. Correlated responses in life-history traits to artificial selection for body-weight in *Drosophila melanogaster*. Evolution 46:745–752.

Hollis, B., and D. Houle. 2011. Populations with elevated mutation load do not benefit from the operation of sexual selection. J. Evol. Biol. 24:1918–1926.

Hollis, B., D. Houle, Z. Yan, T. J. Kawecki, and L. Keller. 2014. Evolution under monogamy feminizes gene expression in *Drosophila melanogaster*. Nat. Comm. 5:3482.

Hollis, B. and T. J. Kawecki. 2014. Male cognitive performance declines in the absence of sexual selection. Proc. R Soc. B Biol. Sci. 281:20132873.

Honek, A. 1993. Intraspecific variation in body size and fecundity in insects—a general relationship. Oikos 66:483–492.

Houle, D., and L. Rowe. 2003. Natural selection in a bottle. Am. Nat. 161:50–67.

Jones, T. M., R. Featherston, D. B. B. P. Paris, and M. A. Elgar. 2007. Age-related sperm transfer and sperm competitive ability in the male hide beetle. Behav. Ecol. 18:251–258.

Kim, D., G. Pertea, C. Trapnell, H. Pimentel, R. Kelley, and S. L. Salzberg. 2013. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14:R36.

Kozlowski, J. 1992. Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. Trends Ecol. Evol. 7:15–19.

Lemmen, J., B. Andrew Keddie, and M. L. Evenden. 2016. Size and protein content of accessory glands in adult male *Caloptilia fraxinella* in different physiological states. Physiol. Entomol. 41:74–82.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87:331–333.

Lüpold, S., M. K. Manier, N. Puniamoorthy, C. Schoff, W. T. Starmer, S. H. B. Luepold, J. M. Belote, and S. Pitnick. 2016. How sexual selection can drive the evolution of costly sperm ornamentation. Nature 533: 535–538.

Maklakov, A. A., T. Bilde, and Y. Lubin. 2004. Sexual selection for increased male body size and protandry in a spider. Anim. Behav. 68:1041–1048.

McMillan, I., M. Fitz-Earle, and D. S. Robson. 1970. Quantitative genetics of fertility. II. Lifetime egg production of *Drosophila melanogaster*—experimental. Genetics 65:355–369.

Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. Annu. Rev. Entomol. 43:63–83.

Nylin, S., C. Wiklund, P. O. Wickman, and E. Garciabarros. 1993. Absence of trade-offs between sexual size dimorphism and early male emergence in a butterfly. Ecology 74:1414–1427.

Pitnick, S., T. A. Markow, and G. S. Spicer. 1995. Delayed male maturity is a cost of producing large sperm in Drosophila. Proc. Natl. Acad. Sci. USA 92:10614–10618.

Poissant, J., A. J. Wilson, and D. W. Coltman. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. Evolution 64:97–107.

Prasad, N. G., S. Bedhomme, T. Day, and A. K. Chippindale. 2007. An evolutionary cost of separate genders revealed by male-limited evolution. Am. Nat. 169:29–37.

Prasad, N. G., M. Shakarad, V. M. Gohil, V. Sheeba, M. Rajamani, and A. Joshi. 2000. Evolution of reduced pre-adult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time. Genet. Res. 76:249–259.

Rice, W. R. 1984. Sex-chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742.

Roff, D. A. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York.

Roff, D. A., and D. J. Fairbairn. 1993. The evolution of alternate morphologies—fitness and wing morphology in male sand crickets. Evolution 47:1572–1584.

Ruhmann, H., K. U. Wensing, N. Neuhalfen, J.-H. Specker, and C. Fricke. 2016. Early reproductive success in Drosophila males is dependent on maturity of the accessory gland. Behav. Ecol. 2016:arw123.

SAS Institute. 2011. The SAS System for Windows, release 9.2. SAS Institute, Cary, NC.

Singer, M. C. 1982. Sexual selection for small size in male butterflies. Am. Nat. 119:440–443.

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, Oxford; New York.

Wiklund, C., and T. Fagerström. 1977. Why do males emerge before females? Oecologia 31:153–158.

Wiklund, C., S. Nylin, and J. Forsberg. 1991. Sex-related variation in growth-rate as a result of selection for large size and protandry in a bivoltine butterfly, pieris-napi. Oikos 60:241–250.

Zonneveld, C. 1996. Being big or emerging early? Polyandry and the trade-off between size and emergence in male butterflies. Am. Nat. 147:946–965.

Zwaan, B. J., W. G. Zijlstra, M. Keller, J. Pijpe, and P. M. Brakefield. 2008. Potential constraints on evolution: sexual dimorphism and the problem of protandry in the butterfly *Bicyclus anynana*. J. Genet. 87:395–405.

Associate Editor: D. Marshall
Handling Editor: M. Servedio

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supporting information S1. Transcriptomic maturity marker genes for males. For each, the Flybase gene ID, $\log_2$ fold change (1 to 4 days of age), and adjusted p value (for the effect of age) are listed, along with the normalized read counts and transcriptomic maturity estimates for each of the six evolved populations.

Supporting information S2. Transcriptomic maturity marker genes for females. For each, the Flybase gene ID, $\log_2$ fold change (1 to 4 days of age), and adjusted p value (for the effect of age) are listed, along with the normalized read counts and transcriptomic maturity estimates for each of the six evolved populations.