

Differentiation of Ovarian Development and the Evolution of Fecundity in Rapidly Diverging Exotic Beetle Populations



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ABSTRACT

Fecundity is a fundamental determinant of fitness, yet the proximate developmental and physiological mechanisms that enable its often rapid evolution in natural populations are poorly understood. Here, we investigated two populations of the dung beetle *Onthophagus taurus* that were established in exotic ranges in the early 1970s. These populations are subject to drastically different levels of resource competition in the field, and have diverged dramatically in female fecundity. Specifically, Western Australian *O. taurus* experience high levels of resource competition, and exhibit greatly elevated reproductive output compared to beetles from the Eastern US, where resource competition is minimal and female fecundity is low. We compared patterns of ovarian maturation, relative investment into and timing of egg production, and potential trade-offs between ovarian investment and the duration of larval development and adult body size between populations representative of both exotic ranges. We found that the rapid divergence in fecundity between exotic populations is associated with striking differences in several aspects of ovarian development: (1) Western Australian females exhibit accelerated ovarian development, (2) produce more eggs, (3) bigger eggs, and (4) start laying eggs earlier compared to their Eastern US counterparts. At the same time, divergence in ovarian maturation patterns occurred alongside changes in (5) larval developmental time, and (6) adult body size, and (7) mass. Western Australian females take longer to complete larval development and, surprisingly, emerge into smaller yet heavier adults than size-matched Eastern US females. We discuss our results in the context of the evolutionary developmental biology of fecundity in exotic populations. *J. Exp. Zool.* 9999A:1–10, 2015. © 2015 Wiley Periodicals, Inc.

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Competition for resources, such as food or nesting sites, plays a critical role in shaping the ecology, population dynamics, and life histories of natural populations (Fisher, '58; Mueller et al., '91; Agnew et al., 2002; Wolf, 2003; Hadfield et al., 2011; Wilson, 2014). In the presence of competition, the expression of a resource-dependent phenotype in any given individual depends upon the degree to which resource acquisition is limited by competitors (Wilson, 2014). As a resource-dependent life history trait, fecundity is expected to be heavily influenced by the degree of competition that natural populations experience in the field, and much evidence exists that reproductive output can vary

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substantially depending on the competitive environment individuals experience either as juveniles (e.g., Credland et al., '86) or adults (e.g., Beckers et al., 2015). Selection via fecundity or mating success tends to be greater than selection via survival, and therefore, fecundity is a fundamental determinant of fitness in natural populations (Kingsolver et al., 2001). However, the proximate developmental and physiological mechanisms that enable fecundity to evolve in natural populations are poorly understood.

Onthophagus beetles rely exclusively on a single resource, dung, for both feeding and reproduction, which makes them excellent models for the study of resource-dependent selection (Moczek, 2003). Adults feed on dung pads and dig tunnels underneath them, provisioning dung for offspring in the form of brood balls at the blind end of each tunnel. Females lay a single egg in each brood ball, and larvae complete their development until metamorphosis feeding exclusively on the dung provisioned in the brood ball (Halffter and Edmonds, '82; Moczek and Emlen, '99). Therefore, competition among adult beetles for fresh dung and tunneling space critically impact the frequency of opportunities for reproduction (Hanski and Cambefort, '91).

The Mediterranean dung beetle *Onthophagus taurus* (Schreber, 1759) was introduced into Western Australia (Tyndale-Biscoe, '96) and the Eastern United States (Fincher and Woodruff, '75) in the early 1970s, resulting in rapid divergence of native and exotic populations following complete geographical separation for approximately 100 generations (Moczek et al., 2002; Moczek, 2003; Kijimoto et al., 2012). Today, the exotic populations of *O. taurus* occur at extremely different densities, resulting in dramatic differences in the intensity of intra- and interspecific competition for breeding opportunities (Moczek, 2003). In the Eastern US (hereafter referred to as EUS), including the state of Indiana, densities of *O. taurus* and other species competing for dung and tunneling space are very low, such that dung pads naturally occurring in this area remain mostly unused. In contrast, Western Australian (hereafter referred to as WA) *O. taurus* and competing species occur at extremely high densities. Most dung pads in this region contain several hundred beetles, and are actively removed from pastures through the beetles' burying activity, at times over the course of hours (Moczek, 2003). This vast difference in population density and associated intensity of competition appear to have resulted in the rapid divergence in average investment into male weaponry used to access females (Moczek, 2003; Buzatto et al., 2012).

Whenever the availability of dung is limited, and therefore opportunities for reproduction are few, it is expected that selection would favor female traits allowing fast and/or efficient use of this resource, ultimately resulting in enhanced fecundity. In particular, high levels of competition for access to dung should favor females that produce brood balls more efficiently, and produce offspring more readily whenever a breeding opportunity arises. Consistent with this prediction, Beckers et al. (2015) found

that, when access to dung is unconstrained, the WA populations of *O. taurus* produce far more brood balls than their EUS counterparts, and have a greater proportion of reproductively active females. Here, we explored the developmental and physiological mechanisms underlying rapid evolutionary divergence in fecundity rates, by investigating whether divergence in reproductive performance is enabled by differential investment into ovarian development and/or timing of maturation. In dung beetles of the subfamily Scarabaeinae, including *Onthophagus*, ovarian maturation occurs entirely after eclosion of the females into the adult stage (Halffter and Edmonds, '82). Immediately after emergence, the ovary is a single unpaired and undifferentiated organ, which differentiates into a basal region containing eggs (i.e., the germarium) and an apical region containing stem cells in which eggs originate (i.e., the vitellarium) after a period of feeding by the newly-emerged female. Once the germarium contains four oocytes at subsequent stages of differentiation, the most basal egg matures, and the female becomes sexually active. After copulation, additional oocytes are made and mature inside the germarium, and the female initiates the production of brood balls and oviposition. At the end of the ovipositing period, the ovary degenerates, and any unviable eggs left in the germarium are reabsorbed (for details see e.g., Englemann, '70; Halffter and López, '77; Tyndale-Biscoe, '78; Tyndale-Biscoe et al., '81; Halffter and Edmonds, '82; González-Megías and Sánchez-Piñero, 2004).

To investigate putative mechanisms of fecundity evolution among exotic *O. taurus* populations, we focused on the early adult stages of ovarian maturation (excluding the degenerating stage). We sought to quantify the dynamics of ovarian maturation, in terms of relative investment into, and timing of, egg production, and potential trade-offs between ovarian investment and the duration of larval development and adult body size and mass. Specifically, we hypothesized that:

- (i) Ovarian maturation is accelerated and investment into egg production is enhanced in high fecundity WA *O. taurus* compared to low fecundity *O. taurus* in the EUS.
- (ii) This accelerated maturation and/or increased investment into ovarian development in WA beetles might trade-off with other life history traits, in particular adult body size and total development time.

MATERIALS AND METHODS

Beetle Collection and Husbandry

The introduction, environmental conditions, and natural history of *O. taurus* in exotic ranges are described in detail in Moczek (2003) and in Beckers et al. (2015). The beetles used in this study were the laboratory-generated offspring of *O. taurus* collected near Busselton (WA, 33.6478° S, 115.3458° E) and near Bloomington, IN (EUS, 39.1622° N, 86.5292° W).

Laboratory-reared beetles were maintained in the lab for several generations as described in Beckers et al. (2015). Parental colonies contained 200–500 individuals with an approximate 50:50 sex-ratio, and densities intermediate to the very low natural densities observed in the field in the EUS (few individuals per dung pad) and the disproportionately higher densities typical of WA populations (several hundreds of individuals per dung pad; Moczek, 2003; Beckers et al., 2015). These colonies were housed in insectaries (54 cm length \times 30 cm height \times 34 cm width) filled up to a half with a moist sand/soil mixture (2:1), and fed twice per week with \sim 0.5 L fresh dung. All beetles were maintained and reared in an environmental chamber at 24°C, 40% humidity, and a light:dark cycle of 16:8 hr.

Experimental Setup—Generating Females for Ovarian Analysis

We compared ovarian development in newly-eclosed females derived from EUS and WA populations. To generate these females, we selected adults at random from the parental colonies (see above), and placed them in breeding containers (Moczek and Nagy, 2005), using 5–7 females and 3–5 males, for a total of 10 individuals per container (EUS: $n=19$; WA: $n=30$ breeding containers). Beetles were provided with unlimited access to dung for an eight-day breeding period. At the end of this period, brood balls were harvested and adult beetles returned to the parental colonies. Brood balls were subsequently incubated in plastic containers (946 mL) that were filled with a sterile soil/sand mixture (2:1) and covered with clear plastic wrap. A small plastic cup (89 mL) was placed in each brood ball container, flush with the soil surface, and served as pitfall trap to collect emerging beetles. We checked these traps for adult beetle emergence daily between 11:30 am and 1:30 pm for 60 days after brood ball harvesting. Newly-emerged adult beetles were removed on the day they were found in the traps (=day 0). Male adults were transferred to a maturation colony (i.e., an insectary set up like the parental colonies—see above), from which they were added to the parental colony after at least 2 weeks to ensure sufficient time for sexual maturation. Females were either put in the maturation colony or weighed to the closest 0.0001 g using a Mettler Toledo (AL 54) scale and then used for experiments.

On day 0, we paired each newly-emerged experimental female with a single sexually mature male (i.e., older than 2 weeks) taken at random from the parental colony of the same population. Previous work has shown that the presence of sexually mature males with newly emerged females is critical for ovarian maturation (Englemann, '70). Specifically, egg production may not proceed beyond the development of the first four eggs in the ovary unless copulation occurs (Halfpter and López, '77; Halfpter and Edmonds, '82). Each pair was placed in a cylindrical, light-impermeable container (Bulk Buys HS027 Jumbo Pasta Keeper: 1500 mL, 27 cm height, 7.2 cm diameter) filled to a height of \sim 20 cm with a moist firmly packed mixture of sterilized soil and sand (2:1 ratio), and provided with \sim 200 g of defrosted

homogenized cow manure (Beckers et al., 2015). Containers were then covered with window screen and perforated black plastic foil to enable ventilation and prevent escape, and beetles were given at least 1 and at most 9 days for breeding (13 ± 2 containers/day per population). For breeding periods longer than 5 days, we replaced the old dung with fresh dung on the fifth day. At the end of each breeding period, we searched the soil and dung in each experimental container for brood balls and beetles, at the same time of the day that we had set up the containers on day 0. Each breeding period was therefore a multiple of approximately 24 hr. Note that only a fraction of WA females produced brood balls (see below), but all of the brood balls retrieved contained one egg. Most of the dung provided in the containers remained unused, indicating that the beetles were not resource-limited during the experiment. All females were fixed and preserved in 70% ethanol after experimental breeding. We excluded all data from containers in which either the male, the female, or both beetles had died during the experimental period.

For each female, we also recorded (1) the duration of immature development, as the number of days between the setup of the parental individuals in breeding containers and the adult emergence of a given female (day 0: see above; this measurement slightly overestimates the duration of development, but it does so equally for both populations); (2) adult mass at emergence, measured to the closest 0.0001 g using a Mettler Toledo (AL 54) scale; and (3) adult pronotum width, measured using a 2D image analysis setup including a stereoscope (Leica MZ-16, Bannockburn, IL, USA), a digital camera (Scion, Frederick, MD, USA), and the software ImageJ (Rasband, '97–2014). Pronotum width and mass at emergence are commonly used proxies for adult body size (e.g., Emlen, '96; Moczek, 2003; Messina, 2004; Vamosi, 2005; Hardersen et al., 2011; Macagno et al., 2011; Beckers et al., 2015).

Assessment of Ovarian Maturation and Investment in Egg Production: Dissection, Imaging, and Measurements

We compared timing and degree of ovarian maturation in WA and EUS females. To do so, the experimental female beetles that were preserved in 70% ethanol (as described above) were rehydrated in ddH₂O for 3 min, and then dissected in phosphate-buffered saline (PBS). Once removed from the abdomen, each ovary was placed in 1 mL dissection dishes filled with a solution of 0.01% Tween20 in PBS, and photographed using the image acquisition equipment described above. We counted the number of eggs in each ovary, and then used ImageJ (Rasband, '97–2014) to digitize the outline of each egg, and measure the outlined area on calibrated images.

In addition, we assessed the degree of ovarian development on a morphological basis, following insights from a series of previous studies on *Onthophagus* and other Scarabaeidae (for details see Tyndale-Biscoe, '78; Tyndale-Biscoe et al., '81; Halfpter and Edmonds, '82; González-Megías and

Sánchez-Piñero, 2004). Specifically, we distinguished four maturation stages (Fig. 1): (1) undeveloped, with no egg visible; (2) partially developed, with two or three small developing eggs; (3) mature, with four or more large eggs in sequential stages of development; (4) degenerated, with 1–2 eggs being reabsorbed, and a yellow body visible at the base of the ovary. Yellow bodies are common markers indicating senescence of the ovary and the end of the reproductive stage (Tyndale-Biscoe et al., '81; González-Megías and Sánchez-Piñero, 2004).

Data Analysis

While females of both populations exhibited signs of ovarian maturation during the experimental period, only WA females oviposited a subset of eggs into brood balls. To incorporate this qualitative difference into our analysis, we split the whole dataset into days before and after these females started laying eggs (days 1–6 and 7–9, respectively). We computed the square root of total egg area in the ovary, divided by each female's body size to normalize the body size-related differences in egg area, counted the total number of eggs produced by each female (i.e., eggs inside the ovary plus eggs laid in brood balls), and compared these data between populations using Mann-Whitney U tests. We used Z-tests to compare populations with respect to (a) the proportion

of females with at least one egg inside their ovary, and (b) the proportion of females with at least four eggs inside their ovary. We analyzed these proportions separately for the period before and after WA females started ovipositing (Table 1). To identify differences in the onset of maturation stages 2 and 3 (see above), we also used pairwise Z tests to compare inter-population differences of these proportions on each day after emergence. We adjusted the significance level for these multiple comparisons using Holm-Bonferroni corrections (Holm, '79).

To evaluate the variation in (1) total egg size in the ovary; (2) average egg size (i.e., total egg size/egg number in ovary); and (3) total egg size standardized for maternal body size (i.e., square root of total egg size in ovary/body size), we used these data as response variables in separate Generalized Linear Models (Gamma distribution probability, log link function) with “days after emergence,” “population,” and the interaction between these two terms as independent variables. The significance of these terms and their interaction was evaluated with Wald χ^2 statistics, and trends were described based on visual inspection of the graphs.

Lastly, we inspected whether differential investment into ovarian development between WA and EUS populations might be associated with differential duration of immature development



Figure 1. The four stages of maturation of the ovary (o) in *O. taurus*. (1) Undeveloped; (2) partially developed; (3) mature; (4) degenerated, with a yellow body (yb) visible at the base. All pictures are taken at the same magnification and report the location of the calyx (c), which connects the ovary to the vagina (v). No experimental female was found to be in stage four (panel 4 portrays the ovary dissected from an old, non-experimental female).

Table 1. Differences between Western Australian (WA) and Eastern US (EUS) *Onthophagus taurus* females in: (a) investment in total egg size in ovary, normalized for body size (median reported); (b) total egg count (eggs in ovary + eggs laid, median reported); (c) % of females with at least one developing egg in the ovary; (d) % of females with at least four developed eggs in the ovary.

	Days 1–6				Days 7–9			
	EUS (n = 71)	WA (n = 78)	Test statistics	P	EUS (n = 39)	WA (n = 38)	Test statistics	P
a sqrt (total egg area)/body size	0.00	0.04	u = 2265.5	0.03	0.10	0.22	u = 435	<0.01
b Total no. eggs	0	1	u = 2383	0.14	3	5	u = 436.5	<0.01
c % Females with 1+ eggs in ovary	49.30	53.85	z = -0.5551	0.57	89.74	97.37	z = -1.3575	0.17
d % Females with 4+ eggs in ovary	2.82	12.82	z = -2.2412	0.02	38.46	71.05	z = -2.8715	<0.01

Values are compared both before (days 1–6) and after (days 7–9) the WA females start laying eggs. Significance is assessed with Mann-Whitney U tests (a, b) and Z-tests for the comparison of sampling proportions (c, d).

and/or investment into adult body size. We compared the duration of immature development, body size, and adult mass at emergence of the experimental females between populations using t-tests. Additionally, we tested for differential scaling between body size and mass between populations by regressing body size (i.e., pronotum width) onto mass at emergence, and comparing the slopes of these regression lines by including the interaction between “population” and “mass” in an ANCOVA. We subsequently removed this non-significant interaction and tested for differences in the intercepts, using a full-factorial model. Prior to running these analyses, we inspected the normal probability plots to confirm the absence of substantive departures from normality, and tested the assumption of homogeneity of variances using Levene’s tests. All statistical analyses were performed using SPSS 22.0.

RESULTS

Timing of Ovarian Development

WA females began to develop eggs, and therefore, entered stage two of ovarian development (i.e., one–three developing eggs; see above), as early as 2 days after emergence, whereas EUS females required at least one extra day (Fig. 2). Likewise, WA females entered stage 3 of ovarian development (marked by the presence of four or more developed eggs in the ovary) one day earlier than EUS females (days 4 and 5, respectively). Within this third stage, on days 7 through 9, we observed a considerable temporary drop and rebound in the percentage of EUS females with four or more developed eggs (i.e., from >50%, to 15%, back to >50%; see Fig. 2), whereas the percentage of WA stage 3 females remained 60% or higher from day 6 through day 9. The proportion of females with four or more eggs in the ovary was significantly higher in WA than in

EUS on day 8 (Pair-wise Z test for the comparison of proportions, $P < 0.01$, Holm-Bonferroni sequential correction applied) and possibly on day 6 ($P = 0.02$ without correction, n.s. with correction). Thus, egg development of WA females started earlier and remained more consistent throughout the experimental time period compared to EUS females.

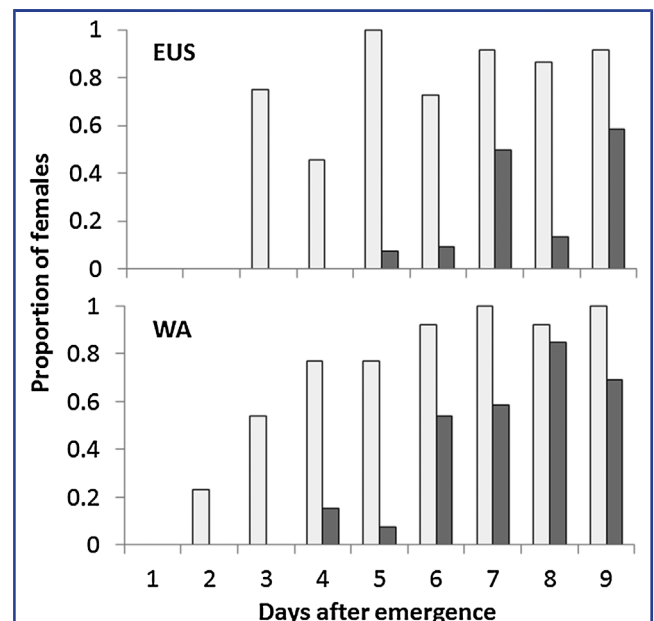


Figure 2. Proportion of *O. taurus* females with at least one (light gray bars) and four developed eggs (dark gray bars) in the ovary on days 1–9 after emergence. Top: Eastern US. Bottom: Western Australia.

Timing of Oviposition

No EUS female oviposited eggs into brood balls during the study period. By contrast, starting with day 7, 20–30% of the experimental WA females oviposited between 2 and 7 eggs each. Importantly, even those females that had oviposited 5–7 eggs still had 4–7 eggs left in their ovaries and no yellow body was detectable at the time of dissection, indicating that these females had not approached the end of their reproductive stage. Again, this result indicates a heightened reproductive output of WA compared to EUS females.

Ovarian Investment—Egg Size and Number

The total egg size in the ovary depended both on the population [Wald $\chi^2(1)=13.382$, $P<0.001$] and the day following emergence [Wald $\chi^2(7)=131.719$, $P<0.001$], while the interaction between these two factors was not significant [Wald $\chi^2(6)=9.543$, $P>0.05$]. As a general trend (see Fig. 3), the investment in egg production (estimated as total size of all eggs in ovary) increased with time, and was more elevated for WA females. Specifically, among WA females, ovarian investment increased markedly between days 5 and 7, and remained high on days 8 and 9. In contrast, among EUS females, investment peaked on day 7, followed by a marked decrease of total ovarian egg size on days 8 and 9. Models carried out with the same factors and interactions, and with [tot egg size/egg number in ovary] and [sqrt(total egg area)/body size] as dependent variables, yielded comparable results (i.e.,

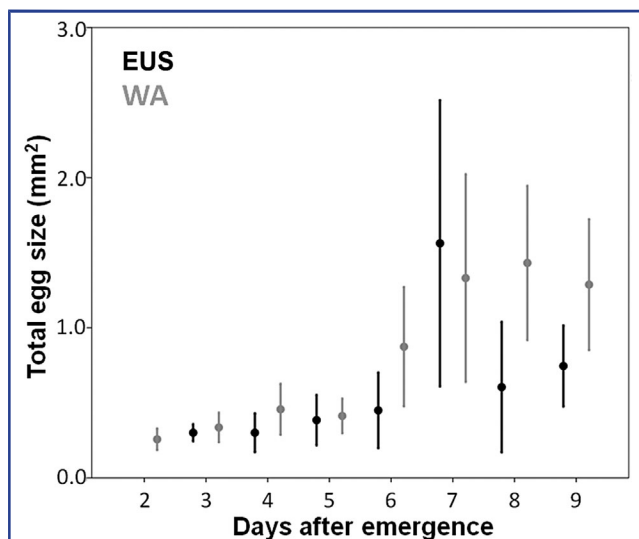


Figure 3. Total egg size in ovary in the nine days following emergence of the experimental females. Means and 95% confidence intervals are reported. Black = Eastern US, gray = Western Australia.

“population”: $P<0.05$; “days after emergence”: $P<0.05$; interaction: $P>0.05$ for both models).

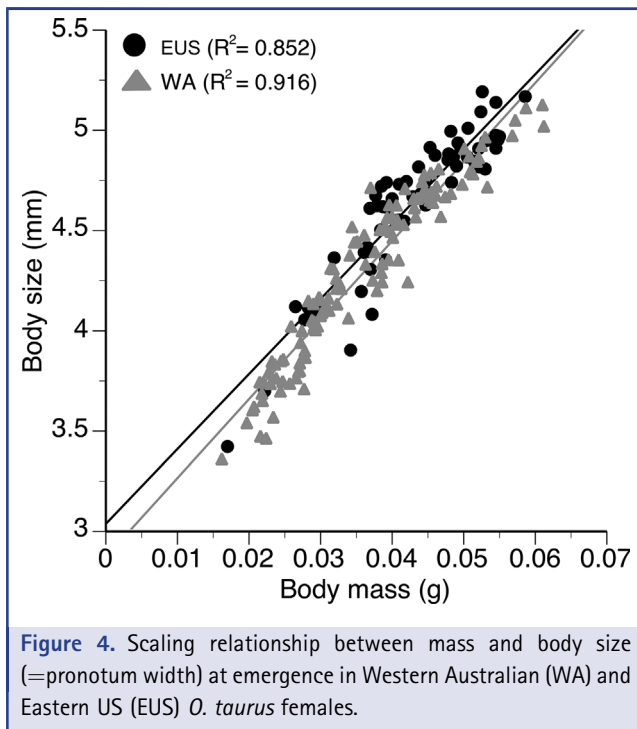
We also analyzed the data separately for days 1–6 (no oviposition by WA females) and days 7–9 (oviposition by WA females). Investment in total egg size in ovaries, normalized to body size, and the percentage of females with at least four developed eggs in the ovaries were both significantly higher in the WA population, both before and after the WA females started ovipositing (Table 1). Similarly, the total number of eggs produced by each female (number of eggs found in the ovaries + eggs laid) was significantly higher in WA females between days 7 and 9 (Table 1). Overall, WA females produced more and bigger eggs than EUS females, especially after WA started ovipositing, but also when neither population was reproductively active.

Duration of Immature Development and Investment Into Adult Body Size

Lastly, we inspected whether differential investment into ovarian development between WA and EUS populations might be associated with variation in the duration of immature development, and/or investment into adult body size. Compared to EUS females ($n=110$), we found that WA females ($n=116$) took ~ 3 days longer to complete immature development (i.e., days between setting up parents to breed until adult female emergence, mean \pm SD: 46.70 ± 3.21 vs. 49.61 ± 4.49 ; T-test: $t(318.335)=-6.823$, $P<0.001$). At the same time, at emergence, WA females were on average both smaller and lighter than the EUS females (pronotum width (mm) mean \pm SD: EUS 4.70 ± 0.40 , WA 4.30 ± 0.43 ; $t(224)=7.077$, $P<0.001$. Mass (g) mean \pm SD: EUS 0.043 ± 0.009 , WA 0.036 ± 0.010 ; T-test: $t(148.88)=4.457$, $P<0.001$). We detected no significant interaction between mass and body size (GLM: “population \times mass”: $P>0.05$), indicating that the slopes of the scaling relationship between these two variables were not different between the two populations (Fig. 4). However, both “population” and “mass” had a significant effect on body size (ANCOVA: $P<0.01$) and the intercept for WA females was significantly lower than for EUS females, indicating that, at any given body size, WA females were consistently heavier than their EUS counterparts.

DISCUSSION

Resource competition is an important driver of diversification within and among species (Pfennig and Pfennig, 2012). Among exotic populations of *Onthophagus taurus*, competition for breeding opportunities appears to have been a critical driver of rapid divergence in diverse morphological (Moczek et al., 2002; Moczek, 2003; Buzatto et al., 2012; Kijimoto et al., 2012) and life history traits, including fecundity (Beckers et al., 2015), in an extraordinarily short time since introduction in the early 1970s. Here, we explored putative developmental and physiological mechanisms underlying rapid fecundity evolution in two of these



populations, as well as their potential correlated consequences. Specifically, we investigated (a) whether exotic *O. taurus* populations that exhibit major differences in fecundity have diverged in aspects of early ovarian development, such as timing and investment into egg production, and (b) if enhanced investment into ovarian development might be correlated with changes in body size and/or the duration of larval development. We found that high resource competition and high fecundity WA *O. taurus* females developed ovaries more quickly, produced more and larger eggs, and started laying eggs earlier than low resource competition and low fecundity EUS *O. taurus* females. Furthermore, on average WA females emerged as smaller adults after a longer larval phase, but at a heavier size than size-matched EUS counterparts. Below we discuss the most important implications of our study.

Differential Early Ovarian Development and Egg Production Enables Rapid Divergence in Fecundity Between *O. taurus* Populations That Differ in Resource Competition

Female traits allowing fast/efficient resource use for reproduction should be favored whenever resource availability is limited, and therefore, windows of opportunity for reproduction are short. In keeping with this prediction, Beckers et al. (2015) found that the Western Australian populations of *O. taurus* produce more brood balls than the Eastern US populations and have a greater proportion of reproductively active females when access to dung is unconstrained, like it was in our experiment. Here, we demonstrate that several aspects of early ovarian development

and egg production enable the higher reproductive output of WA females. Specifically, we found that Western Australian females, which in the wild experience severe resource competition (Moczek, 2003), (1) start developing eggs and fully complete ovarian maturation at least 1 day earlier than low fecundity EUS females; (2) invest significantly more into overall egg size and number; and (3) start producing brood balls containing viable eggs as early as 7 days after emergence, whereas the EUS females hold off reproduction until at least day 9.

Ovarian maturation differed remarkably between females of the two populations not only in the onset of several key-stages, but also with respect to the overall pattern. In the WA population, total egg size in the ovary consistently increased from day 5 to 7 after emergence, and leveled out for the remaining experimental days. In contrast, in the EUS population total egg size peaked on day 7 (with a large variance, possibly due to a bimodal distribution of egg size), while dropping considerably during the following days (Fig. 2). At the same time, between day 7 and 8 there was a sizeable drop in the proportion of EUS females with at least four developed eggs in the ovary. Combined, these observations suggest that even though several EUS females are potentially ready to reproduce as early as day 7, contrary to the WA females they commonly opt not to oviposit in brood balls at this point, and instead either reabsorb or otherwise dispose of the first egg in the ovary, possibly by eating it. Egg absorption and consumption have both been reported in insects (Bell and Bohm, '75; Polis, '81). Since we did not detect any yellow bodies in the dissected ovaries, we believe reabsorption at this stage might be unlikely, though determining reliably whether either process actually occurs in EUS *O. taurus* will require future work. Overall, our results suggest that divergence in fecundity between WA and EUS *O. taurus* females is related to differential ovarian development of early adults, and by extension may evolve surprisingly rapidly in geographically isolated populations that differ substantially in resource competition.

Intriguingly, companion studies investigating the transcriptome dynamics across larval and adult development of WA and EUS males and females further support this conclusion, and hint at possible endocrine and genetic targets of selection (Pespeni and Moczek, in review). Specifically, 4-day old adults whose transcriptomes were sequenced using RNAseq exhibited striking differences in expression of Juvenile Hormone Esterase (JHE) between populations: JHE was upregulated in 4-day old adult females from the Eastern US, but not the corresponding males, nor same-aged males and females from WA, nor any male or female individuals sampled from earlier or later developmental stages. In adult insects, Juvenile Hormone (JH) stimulates follicle maturation and the production of eggs, whereas JHE degrades JH, thereby inhibiting egg production (Nijhout, '98). This preliminary finding, therefore, suggests a potential proximate candidate mechanism for the differentiation of ovarian maturation via

differential, population-specific inhibition of early egg development in newly emerged *O. taurus* females. Further studies are needed to explore this intriguing possibility.

Higher Fecundity Is Associated With Reduced Body Size in WA *O. taurus*

Compared to EUS females, WA females were smaller, yet surprisingly, required significantly longer to complete larval development, in contrast to the general expectation that smaller individuals of the same species should take less time to complete development (Roff, 2002; Davidowitz et al., 2004; Kingsolver et al., 2012). In insects, emergence at a smaller body size is frequently associated with reduced fitness (e.g., Allen and Hunt, 2001; Kolluru and Zuk, 2001; Kingsolver and Huey, 2008), and population growth rate is slowed if developmental and thus generation times are longer (Kingsolver and Huey, 2008). However, the high fecundity observed in WA females (Beckers et al., 2015) contradicts the general rule that small size equals reduced fitness. At the same time, we also found that WA females emerge as heavier adults compared to their size-matched EUS counterparts (Fig. 4). In other words, the WA females emerged “denser” than equal-sized EUS females, possibly through the elevated accumulation of fat body during larval development. Since the energy accumulated in the form of fat body is essential for reproduction (Arrese and Soulages, 2010), this may explain why WA females require longer to complete larval development, yet emerge as smaller but heavier individuals, ready to reproduce more quickly and at a higher rate than their same-size EUS counterparts. More generally, our results raise the possibility that reduced body size and lengthened larval developmental periods constitute a byproduct of the elevated ovarian maturation detected in high fecundity WA populations of *O. taurus*. Future work is needed to confirm causal connections between these observations.

Divergence in Ovarian Development: Alternative Explanations

Differences in ovarian development detected between EUS and WA populations could alternatively be the product of neutral divergence following founder effects dating back to introduction into the two exotic ranges (Moczek et al., 2002). While we cannot fully exclude this possibility, it is worth highlighting that the suite of co-diverging traits identified in this as well as previous work (Beckers et al., 2015) appear likely adaptive to WA females, as they would enable these females to reproduce more efficiently in an extremely competitive environment, whereas delaying reproduction is unlikely to carry significant fitness costs for the EUS population given the unconstrained availability of breeding opportunities in this region. Estimating live-time fitness in field conditions, ideally combined with transplant experiments, would be needed to further resolve these issues.

It is also possible that differences in ovarian maturation patterns and female fertility are at least in part male-mediated,

rather than dependent solely on female-specific trait divergence. Specifically, since ovarian maturation is well-known to be enhanced by copulation (Englemann, '70; Halffter and López, '77; Halffter and Edmonds, '82), a consistent delay in fertilization could explain delayed egg development in the EUS population. Indeed, this possibility is supported by the observation that the first eggs of EUS females are likely reabsorbed or consumed (see above), which is more common for unfertilized than for fertilized eggs (Bell and Bohm, '75; Polis, '81). Similarly, it is tempting to speculate that differential expression of JHE may be dependent on fertilization, in a way that mating may lead to a suppression of JHE levels, causing any delay in fertilization to also delay ovarian development. Further studies are clearly needed to explore these hypotheses.

CONCLUSIONS

Our results suggest that divergences in fecundity between exotic, recently established populations of *O. taurus* have evolved through differentiation of several aspects of early ovarian development. At the same time, we found that divergence in ovarian maturation patterns occurred alongside correlated changes in duration of larval development, body size, and mass. These findings raise the possibility that rapid divergences in fecundity may be enabled by developmental mechanisms that force other life history and morphological traits to diverge as a byproduct, providing several avenues for correlated evolution in directions that might or might not be adaptive. Intriguingly, Juvenile Hormone and Juvenile Hormone Esterase, which ongoing investigations suggest as candidate factors in the differentiation of ovarian maturation in exotic *O. taurus* populations, are also key mediators of trade-offs across diverse life history traits in a number of insects (*Drosophila*, grasshoppers, butterflies, and beetles: Flatt et al., 2005). Further studies addressing the developmental mechanisms that underlie variation and integration of life history traits are needed to advance our understanding of the evolutionary diversity of life cycles in natural populations of onthophagine beetles and beyond.

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