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**Laboratory maintenance does not affect macro-ecological and macro-physiological
patterns: A *Drosophila* case study**

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Abstract:

Large comparative studies in animal ecology, physiology, and evolution often use animals reared in the laboratory for many generations; however, the relevance of these studies hinges on the assumption that laboratory populations are still representative for their wild living conspecifics. In the present study, we investigate if laboratory-maintained and freshly-collected animal populations are fundamentally different and if data from laboratory-maintained animals are valid to use in large comparative investigations of ecological and physiological patterns. Here, we obtained nine species of *Drosophila* with paired populations of laboratory-maintained and freshly-collected flies. These species, representing a range of ecotypes, were then assayed for four stress tolerance traits, two body size traits, and six life history traits. For all of these traits, we observed small differences in species-specific comparisons between field and laboratory populations; however, these differences were unsystematic and laboratory maintenance did not eclipse fundamental species characteristics. To investigate if laboratory maintenance influence the general patterns in comparative studies, we correlated stress tolerance and life history traits with environmental traits for the laboratory-maintained and freshly-collected populations. Based on this analysis we found that the comparative physiological and ecological trait correlations are largely similar irrespective of provenience. This finding is important for comparative biology in general because it validates comparative meta-analyses based on laboratory-maintained populations.

Key Words: laboratory adaptation, critical thermal minimum, critical thermal maximum, standard metabolic rate, fecundity, developmental time

Introduction:

Laboratory populations of several invertebrate taxa including moths, mosquitos, beetles, marine copepods, and fruit flies have been used to study aspects of evolution, ecology, physiology, and biochemistry for decades. This abundance of data is often compiled and used in large comparative studies in order to find general biological patterns. Accordingly, ecologists may rely on data from laboratory populations when studying the relation between environmental variables and phenotypic variance (Addo-Bediako *et al.*, 2000; Chown *et al.*, 2002; Kellermann *et al.*, 2009, 2012a; b). Several large-scale comparative studies and meta-analyses have used such data to infer aspects of species' responses to climate change (Deutsch *et al.*, 2008; Sunday *et al.*, 2011; Diamond *et al.*, 2012; Araújo *et al.*, 2013). Moreover, physiologists have frequently utilized comparisons of laboratory-maintained species when investigating temporal and spatial variation in fitness components in order to understand the mechanistic basis of tolerance and life history traits (Goto & Kimura, 1998; Chown *et al.*, 2004; Nyamukondiwa *et al.*, 2011; Chown, 2012; Slotsbo *et al.*, 2015). The ecological relevance of this practice has been questioned (Frankham, 1995; Gibbs, 1999; Harshman & Hoffmann, 2000) as the laboratory environment can differ considerably from the species' natural conditions and the genetic variability of laboratory stocks may not represent that of their wild relatives.

Founder effects, genetic drift, inbreeding, and laboratory adaptation are well established phenomena that can cause considerable population differences when comparing to the outbred field populations (Harshman & Hoffmann, 2000; Terblanche & Chown, 2007; Simões *et al.*, 2008; Bechsgaard *et al.*, 2013). Laboratory populations may be established with relatively few specimens and kept at low effective population sizes, resulting in strong genetic drift and inbreeding. Laboratory conditions, both biotic and abiotic, are likely different from those occurring in the natural environment resulting in exposure to different types of selection pressures (Harshman & Hoffmann, 2000; Hoffmann *et al.*, 2001). Unfortunately, it is often very difficult to partition the relative contributions of genetic drift, inbreeding, and laboratory adaptation when using existing stock populations (unless the stocks have been created specifically for the purpose of investigating these factors).

To identify if general, directional patterns characterize laboratory populations relative to freshly-collected field populations, we first conduct a review of the literature for a range of invertebrate taxa. Several studies have highlighted the differences between laboratory

populations and freshly-collected populations of invertebrates (Gibbs, 1999; Harshman & Hoffmann, 2000; Terblanche & Chown, 2007) and with this literature survey we are able to ask if the response to the laboratory environment generally weakens stress resistance, changes body size, or alters life history traits across or within taxonomic groups. Secondly, we examine this question experimentally. We do this by comparing a range of traits in a number of *Drosophila* species, each represented by a field and laboratory population originating from the same locality. *Drosophila* were chosen because they represent a popular model insect systems in ecology, evolution and physiology (Barker *et al.*, 2013). Most *Drosophila* species are easy to maintain in the laboratory allowing for true common garden experiments wherein potentially confounding effects (e.g. age, reproductive status, and environmental factors) can be controlled. The popularity of *Drosophila* has resulted in numerous laboratory stocks of multiple species and these stocks provide material for many researchers who have neither the time nor the resources to collect fresh samples for each experiment. It is therefore important to validate the use of laboratory stocks in comparative studies.

Considering the well documented effects of inbreeding, genetic drift, and laboratory adaptation at the intraspecific level, we designed an experiment to test if use of laboratory populations compromise the comparative interspecific approach taken in many ecological and physiological studies (Goto & Kimura, 1998; Addo-Bediako *et al.*, 2000; Marron *et al.*, 2003; Deutsch *et al.*, 2008; Kellermann *et al.*, 2012b; Araújo *et al.*, 2013). We located stock populations for nine species of *Drosophila* for which we could also obtain a freshly collected population of similar geographic origin. We then experimentally compared stress tolerance, body composition, and life history traits in the species-pairs. Because we had limited control over the genetic composition or number of generations kept in the laboratory for the “lab” population (as is commonly the case when using laboratory-maintained populations) we were not designing our experiment to specifically partition the effects of laboratory adaptation, inbreeding or genetic drift. The aim in the present study was to provide a large dataset to examine if laboratory maintained populations were characterized by general trends across a broad range of species and if such differences affected physiological and ecological patterns. With these data, we addressed the following two questions: 1) Are data from laboratory-maintained animals informative when asking ecological and physiological questions in comparative biology? 2) Are laboratory-maintained and freshly-collected populations fundamentally different?

Materials and Methods:***Study organisms:***

Laboratory populations of *Drosophila* were obtained from local collections, through colleagues, or ordered from stock centers (Table 1, for maintenance history and specific collection sites see Table S1). In establishing and comparing the experimental populations we chose to establish fresh mass-bred field populations to make as equal a comparison as possible to the existing mass-bred laboratory populations. In order to conduct a true common garden experiment the adults were placed on standard *Drosophila* medium (Kristensen *et al.*, 2015) and allowed to complete one generation at 19°C. Eggs were collected from the offspring of the initial populations and placed in density controlled vials of ~40 eggs per vial. The adults that emerged were used in the experimental assays when females reached an age where they started to lay fertilized eggs. Using this approach, we were able to partially control for effects of age, diet, and rearing density as the flies experienced two generations in a common environment (common garden approach). In some cases, we received the field population 1-2 generations after collection and could therefore only test the flies at the third or fourth generation (Table 1, Table S1). We acknowledge that some laboratory adaptation may have taken place during this time. We also emphasize that the present study tests only one population of each species. The absence of replicate populations limits our ability to pinpoint the physiological or evolutionary mechanisms responsible for the observed differences between populations (Garland Jr & Adolph, 1994). A more comprehensive approach would be to control for both the number of generations and number of founders in several replicate populations of multiple species (see Sgro & Partridge, 2000) or to test several isofemale lines rather than a mass bread population. However, the main purpose of this study was to evaluate the relevance of laboratory stocks which are typically kept as mass bread populations. Importantly, for all of the experimental assays described below, each species pair (field/lab) were assessed in the same trial using flies of the same age to ensure that the intraspecific comparisons were not affected by block design, age effects or other confounding experimental effects.

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Environmental variables:

In order to determine the usefulness of laboratory populations for ecological studies, we correlated trait values with abiotic environmental variables. These values were obtained from the mean conditions for a given species; specifically estimates of latitude, precipitation, and annual temperature for data covering the whole geographical distribution of each species (see Kellermann *et al.* 2012b for details).

Tolerance traits:

For all of the tolerance traits, we used 20 sexually mature females from both populations of each species. The flies were sorted by sex under brief CO₂ anesthesia and allowed two days of recovery prior to experimentation.

Thermal limits (CT_{min}/CT_{max}): Individuals were placed in 5 mL glass vials in a circulating water/glycol bath at 20°C. Flies were then exposed to a slow ramp of 0.1°C per minute (down or up for assessment of CT_{min}/CT_{max}, respectively) and the temperature at which any movement ceased was recorded the critical thermal limit (CT_{min}/CT_{max}, respectively) for that individual.

Desiccation: Individuals were placed in 5 mL glass vials covered with gauze. Vials were placed in a humidity-controlled chamber (70 cm x 35 cm x 35 cm glass aquarium containing granular silica dioxide with an atmosphere of < 10% RH) and monitored every 2 hours. The time at which all movement ceased was recorded as the desiccation tolerance in hours for a given individual.

Starvation: Individuals were placed in vials containing 2 mL of 2% agar medium at 19°C. This medium was supplied to provide moisture while starving. Vials were monitored every 8 hours and the time at which all movement ceased was recorded as the starvation limit in hours for that individual.

Body composition:

Size and lipid composition: 6 replicates of 10 sexually mature female flies were transferred to Eppendorf tubes and frozen at -20°C. Measurements represent estimates of average wet mass, water and lipid content. Wet mass was measured to the nearest microgram (using a MSE6.6S Sartorius, Goettingen, Germany). To obtain dry mass, flies were weighed again after drying 48 h at 60°C. Water content was calculated as (wet mass-dry mass)/wet mass. Total lipid content was measured in the dried flies after lipid was removed in a Soxhlet apparatus. After lipid removal, the samples were dried for 24 hours and weighed again to determine lipid free dry mass, and thus lipid content.

Life history traits:

Fecundity and developmental time: 15 mated pairs of flies were placed in vials (one pair per vial) with 7 mL of standard media. Each pair was transferred to a fresh vial every second day for 12 days (resulting in 6 vials per pair). The vials were monitored daily to register the time of first adult emergence in each vial. Developmental time for a species/population was averaged across the six vials and reported as the average of the 15 pairs. Fecundity and developmental time data on *D. hydei* were excluded from the analysis due to their failure to reproduce in solitary conditions despite repeated attempts.

Life span: 10 replicates of 10 mated females (100 flies in total) were placed in vials with 3 mL standard media. Every fourth day, the flies were transferred to vials with fresh media and the number of dead flies counted. We calculated median life span for each vial and reported lifespan as the mean of the median of the 10 replicates.

Activity: Activity level was recorded as the proportion of time spent spontaneously moving in a novel environment as described by Rohde *et al.* (2016). Specifically, 18 flies from each population were loaded into a behavioral arena consisting of 36 individual areas that were each 16mm in diameter and 6mm high. The arena was placed on top of a lightbox and, after a 5-minute acclimation period, the flies were recorded for 15 minutes. The resulting videos were analyzed to determine the proportional time spent in motion for each individual using EthoVision XT (Noldus, Wageningen, The Netherlands).

Metabolic Rate/ Q_{10} : The standard metabolic rate (SMR) of fly populations was estimated from rates of CO₂ production. Measurements were obtained using the equipment, methodology and protocol described in detail in Jensen *et al.* 2014. Briefly; intermittent closed respirometry was measured over a 22-hour period using an automated system that sequentially measures CO₂ production rate in 16 parallel respirometry chambers (Sable Systems, Las Vegas, USA). Measurements were obtained by alternating each chamber between a closed condition (where CO₂ accumulates) and an open condition (where the accumulated CO₂ is measured). Each replicate (respirometry chamber) contained 8-10 flies and sequential measurements of CO₂ production were obtained for each closed period (30 and 45 minutes for flies at 20 and 10°C, respectively). The repeated measurements from each chamber allow us to exclude measurements where metabolism is elevated due to activity (See validation and discussion of method in Jensen *et al.*, 2014). Thus, SMR for each species/population was approximated from the mean of 3 lowest measures of CO₂ production rate in each replicate. SMR was measured at 10 and 20°C on

two consecutive days using different batches of flies. Dry masses of flies were obtained after the measurements to report metabolic rate as $\mu\text{l CO}_2 \text{ mg}^{-1} \text{ h}^{-1}$ at 10°C and as Q_{10} ($\text{SMR}_{20^\circ\text{C}}/\text{SMR}_{10^\circ\text{C}}$).

Analyses:

Trait correlations: Macro-physiological patterns are often observed through the study of trait correlations. To test if laboratory populations are characterized by the same trait correlations as field populations we ran two multiple-correlation analyses. Specifically, we correlated the relationship among all twelve measured traits in the field populations and extracted the correlation coefficients. Then we repeated this analysis for the laboratory populations and then analyzed the resulting correlation coefficients with a linear model to test the relationship between the field and laboratory correlation coefficients (for example: is the correlation between starvation tolerance and lipid content similar between field and laboratory populations, etc.). We note that this analysis does not explicitly test the variance of the correlations and refer to the analysis of the individual traits for a discussion of this variance component (See below). Using a similar methodological approach, we tested if macro-ecological patterns were preserved. Here we correlated the measured trait values to a selection of environmental variables for each species (annual mean temperature, annual precipitation and latitude). Subsequently, we examined if similar macro-ecological associations were found for field and laboratory populations. We caution that this analytical approach does not directly examine if similar correlations found within the lab and field populations are bound by the same causal mechanism, nor does this analysis explore in detail the variance within each of these correlations. All analyses were conducted using the R (v3.3.2) statistical program (R Core Team 2014).

Field versus laboratory populations: All traits were analyzed in three ways. First, a pairwise comparison on each species for each trait using a Kruskal-Wallis test to test the significance of the difference in trait values between field and laboratory populations. In running multiple pairwise tests without applying a correction, we run the risk of Type I error, but deemed this approach to be the most conservative and directly comparable to previous studies. Second, to examine the general pattern of population differences between species pairs, we correlated the mean trait value for each laboratory population against the mean value for each field population to quantify the slope and R^2 . Finally, to determine the relative importance of population differences relative to species differences we used linear models including species, population

(field or lab), and interaction thereof, to determine the significance and relative importance of each predictor. We treated population as a categorical variable because the number of founding isofemales and the number of generations in the laboratory are confounded by species. This allowed partitioning of the differences between species and within species associated with laboratory maintenance generally.

Results:

Survey of the literature: Prior to our experimental study, we searched the literature to see if consistent and directional responses were associated with long term laboratory maintenance in insects. Specifically, we searched using the terms: “wild populations”, “field and laboratory”, “wild and laboratory”, “lab adaptation”, and “population comparison”. We limited our scope to invertebrates and excluded studies that only measured insecticide resistance or explicit genetic diversity. This resulted in 28 relevant studies on 7 orders and 77 traits in total. We then partitioned traits into four categories; stress tolerance, size, life history, and behavioral traits. For each trait, we recorded if the study found a significant difference between the newly established “field” population and the laboratory stock population and the direction of that difference relative to the field population.

Data from the literature failed to reveal general patterns of difference between paired populations in stress tolerance, size, life history, or behavioral traits (Fig 1, Table S1). For example, while some studies argue that laboratory maintenance will increase reproductive output and decrease the age at first reproduction (Rössler, 1975; Colloff, 1987; Pité, 2000; Simões *et al.*, 2008), several studies fail to find this pattern or even produces the opposite (Mullin & Brooks, 1967; Nagarkatti & Nagaraja, 1978; Nowosielski-Slepowron & Aryeetey, 1980). Similar ambiguity exists when we consider comparisons of *Drosophila* populations (Fig. 1 B). For example, some studies argue that laboratory maintenance leads to reduced resistance to desiccation and starvation (Hoffmann *et al.*, 2001; Simões *et al.*, 2008), while others report no change or even an increase in these traits (Pité, 2000; Hoffmann *et al.*, 2003; Griffiths *et al.*, 2005). It is unclear if the lack of generality across species and traits is the result of limited available data or differing experimental approaches between studies (Ackermann *et al.*, 2001).

Trait correlations: Here we modeled the correlation coefficients between traits measured in laboratory populations as function of the correlation coefficients between traits measured in the field populations (Table S3) to determine if the direction and strength of the trait-trait relationships are preserved. For all of the trait-trait correlations, the values for field and laboratory populations are tightly correlated ($F_{1,64} = 73.56$, $P = 3.09 \times 10^{-12}$) (Fig. 2A). We note there is some variability in the weaker trait relationships (when $-0.5 < R < 0.5$). However, when the correlations are strong (R exceed ± 0.5) in the field populations they remain strong and with similar direction in the laboratory populations. When we model the relationship between traits as the response variable as a function of population (field or lab) and the types of traits being contrasted (body composition, life history, tolerance, and the combinations thereof) there is no significant difference between field and laboratory trait correlation values ($F_{1,194} = 0.11$, $P = 0.74$). These results suggest that the macro-physiological relationships between traits remain largely intact despite laboratory maintenance.

To consider the correlation between traits and environmental variables, we calculated the R -value of each of the measured traits and environmental variables corresponding to each species (specifically latitude, annual mean temperature, and annual precipitation), for each population (field and lab, Table S3). We modeled the resulting correlation coefficients for field and laboratory populations to test if the direction and strength of trait correlations are preserved (Fig. 2B). The relationship for both field and laboratory populations between trait values and environmental variables is largely unchanged by time in the laboratory (population- $F_{1,66} = 0.002$, $P = 0.96$). Again, these results suggest that the macro-ecological relationships between traits remain largely intact despite laboratory maintenance.

Field versus laboratory populations:

Pairwise comparisons between laboratory-maintained populations and freshly caught populations of the same species often revealed significant differences in trait-values (Fig. 3-5 and Table S4). Importantly, however, most traits were not characterized by consistent and directional differences between the field and laboratory populations. In the following, we briefly emphasize the main results from correlations between field and laboratory populations for the 12 traits examined. For simplicity, these traits have been divided into three somewhat arbitrary categories: tolerance, body composition, and life history traits.

We describe the relationship between trait means of lab and field populations in-terms of R^2 , slope, and relative position to the line of unity. If all species were unaffected by laboratory maintenance, the slope of the relationship between field and lab trait values would lie on the line of unity, and be equal to one. If laboratory maintenance drives convergence of all species to a singular phenotype, then the slope of the relationship would be nearer to zero. Moreover, if all trait types responded in a similar way, all traits would show similar slopes and R^2 (Fig. 3-5).

Tolerance traits:

Estimates of CT_{min} and CT_{max} were very similar between field and laboratory populations (Fig. 3 A&B, $R^2 = 0.98$ for both). In contrast, we found that both desiccation and starvation tolerance have relatively low R^2 values (Fig. 3 C&D, $R^2 = 0.38$ and $R^2 = 0.55$ respectively) even though the correlations are still highly significant and positive (desiccation: $F_{1,7} = 5.98$, $P = 0.04$, slope = 0.67, starvation: $F_{1,7} = 11.08$, $P = 0.01$, slope = 0.83). As seen in figure 3 (and Table S4), we found no consistent directional pattern in any of these tolerance traits (i.e. we do not find that lab maintained populations are consistently characterized by lower or higher trait means).

Body composition: Field flies were generally larger ($F_{1,7} = 63.99$, $P = 4.09 \cdot 10^{-12}$, slope = 0.82) but laboratory flies typically had higher percent lipid ($F_{1,7} = 6.40$, $P = 0.04$, slope = 1.40) (Fig. 4B). However, for both traits there are species that exhibit significant differences between populations in the opposite direction (noted in Table S4).

Life history traits: While there was a great deal of variation in fecundity ($R^2 = 0.43$) the overall trend was in the direction of a decrease in fecundity in the laboratory populations (Fig. 5A, slope = 0.62, $F_{1,7} = 2.04$, $P = 0.15$). While both development time (Fig. 5B, $F_{1,7} = 699.65$, $P = 8.04 \cdot 10^{-4}$, slope = 0.99) and longevity (Fig. 5C, $F_{1,7} = 11.93$, $P = 0.02$, slope = 0.68) were generally higher in laboratory populations, longevity shows a higher degree of variability ($R^2 = 0.578$) with most, but not all of the points lying above the line of unity. Both resting metabolic rate at 10°C and Q_{10} have relatively low R^2 values (Fig. 5D, $R^2 = 0.25$ and Fig. 5E, $R^2 = 0.52$ respectively) and showed no consistent significant directional pattern. Interestingly, activity was generally decreased for laboratory populations (Fig. 5F, $F_{1,7} = 38.91$, $P = 8.31 \cdot 10^{-10}$, $P = 0.01$, slope = 0.5). Moreover, there is a high correlation between activity in field and laboratory populations ($R^2 = 0.70$).

Despite a high degree of variance for some traits (statistics reported in Fig. 3-5) we find that for all the 12 traits measured there is a significant correlation ($P < 0.05$) between field and laboratory populations. This suggests the differences between species are greater than the

differences between populations (field vs. lab). This is further supported by the linear models for each trait where species is always a significant predictor that consistently explains a large relative proportion of the variance in the dataset (Table 3).

Discussion:

Are physiological and ecological patterns maintained in laboratory populations?

Despite the popularity of comparative studies, the validity of using laboratory populations to investigate ecological and eco-physiological patterns is periodically questioned in the literature (Gibbs, 1999; Harshman & Hoffmann, 2000; Terblanche & Chown, 2007). Here we initially made a survey of previous studies that have quantified differences between laboratory-maintained and freshly collected populations. The results of this “mini-review” highlighted the lack of a general response to laboratory maintenance (Fig.1) suggesting that the effects of laboratory maintenance, founder effects and inbreeding are hard to predict.

To our knowledge, there has been no previous multi-species multi-trait study to investigate specifically if laboratory maintenance alters comparative patterns. In the present study, we find that field and laboratory populations of flies are often characterized by some phenotypic differences. However, the intraspecific differences are rarely systematic between species and population differences are relatively small in comparison to species differences. Here we use these data to discuss whether we can use laboratory populations when asking comparative questions in ecology and physiology. Specifically, we use our data to investigate if similar correlations were found between traits within the field and the laboratory populations, respectively.

First, we investigated physiological correlations (for example, the correlation between desiccation tolerance and body-size across species). Using the correlation coefficients from laboratory and field flies, respectively, we tested if trait correlations were similar among the two population groups (Table S3, Fig. 2A). The linear model relating field and laboratory trait correlations shows a strongly positive and significant relationship. This demonstrates that laboratory maintained populations generally have similar trait correlations as the field populations. Importantly the linear model relating trait correlations of field and laboratory populations also highlights considerable variability (Fig. 2A). This variability suggests that physiological correlations can be sensitive to the conditions associated with laboratory

maintenance and that correlations among traits are slightly weaker when using laboratory populations. Nevertheless, laboratory maintained populations generally generate the same types of trait correlations as freshly caught populations and, while both introduce variance, this can be minimized experimentally by including a sufficient number of species if the comparative study is based primarily on laboratory stocks.

To address the validity of using laboratory maintained animals for ecological questions, we repeated this analysis but instead correlated traits to published environmental variables (Kellermann *et al.*, 2012b) (example: the correlation between desiccation tolerance and mean annual precipitation). We found that the relationship between trait values and an environmental variable is unchanged by laboratory maintenance (Fig.2B). The strength of the correlation to environmental traits, which are consistently important in both correlative and mechanistic studies (Deutsch *et al.*, 2008; Kellermann *et al.*, 2009, 2012b), confirms that using laboratory populations in comparative physiological or ecological studies is a valid approach.

Stress tolerance:

Laboratory conditions for *Drosophila* are unnatural; temperatures are constant, there is only one source of readily available food, and the humidity is relatively high. Moreover, it is possible that some laboratory adaptation may have occurred in our field populations as we did not test them directly from the field. Previous studies have suggested that tolerance to stressful conditions will decrease under laboratory conditions (Hoffmann *et al.*, 2001; Griffiths *et al.*, 2005) but here we only find partial support for this claim. In the case of heat tolerance this is perhaps unsurprising given that other studies found no significant difference in heat tolerance or Hsp70 expression of freshly collected and laboratory maintained *D. melanogaster* (Krebs *et al.*, 2001). We observed more substantial differences in desiccation and particularly starvation tolerance between field and laboratory flies (Fig. 3C and D). This idiosyncratic pattern is consistent with the conclusion drawn from the literature (Harshman & Hoffmann, 2000; Griffiths *et al.*, 2005) and suggest that much of variation in desiccation found between populations could be associated with random genetic drift or founder effects (Simões *et al.*, 2008) rather than a general decrease in desiccation tolerance associated with laboratory maintenance.

Body composition and laboratory maintenance:

Previous studies report that time in the laboratory generally increases energy storage in the form of lipids (summarized in Harshman & Hoffmann 2000). A similar tendency was found in four of

the nine species in our study (Fig.3B). Importantly, however, this is not a uniform response. Two of the nine species were characterized by a considerable decrease in lipid content associated with laboratory maintenance. Our data confirm the trend observed in the literature, namely that body size may decrease with laboratory maintenance (Spates & Hightower, 1970; Linnen *et al.*, 2001). Taken together laboratory populations of flies tend to be smaller and have higher lipid content. Interestingly the observed changes in lipid content do not always translate into increased starvation tolerance (compare species specific responses of Fig. S3D and 4B, Table S4).

Life history traits:

Laboratory maintenance may change selective pressure on developmental time, early reproductive output, and life span (see references below). Overall, developmental time was one of the most tightly correlated traits between field and laboratory flies with a slope of 1 and a R^2 of 0.99. Our measures of fecundity revealed considerable variability in species' responses to laboratory maintenance (Fig. 5A). We were able to confirm the findings from a previous study that fecundity increased in laboratory reared *D. melanogaster* (Sgro & Partridge, 2000) but failed to find a significant change in *D. subobscura* although this has also been reported previously (Pité, 2000; Simões *et al.*, 2008). Here we find a strong tendency (significant in six of nine species) for laboratory populations to live longer than field populations (Fig. 5C). Thus, increased longevity is the closest our study came to a general effect of laboratory maintenance.

We had no *a priori* expectations in regards to changes in standard metabolic rate with laboratory adaptation and could only find one previous study that had reported a small increase for *D. melanogaster* in metabolic rate following 100 generations in the laboratory (Berrigan & Partridge, 1997). We only found a significant increase in SMR for the laboratory population of one species while four species showed a significant decrease. We also noted that this trait showed considerable variation among species (Fig. 5D). Similar variability was also observed in Q_{10} of SMR, but this was random and only significant for one species (Fig. 5E). For most species, we found the field and laboratory populations to have similar levels of activity (Fig. 5F). Here we chose to measure spontaneous activity as total distance moved in a circular arena over a 15-minute period. For the two most active species (*D. melanogaster* and *D. hydei*), the laboratory population showed large reductions in activity level (see Table S4 for details). While it is possible that this pattern may reflect relaxed selection for high activity in the laboratory, further

studies are needed to validate this assertion. Furthermore, there is little evidence from our literature search that this trend is universal (Fig. 1).

In conclusion, life history traits show no evidence for general and directional differences between laboratory and field populations. For individual species, phenotypic differences are observed for some life history traits and we did not design our experiment to partition the relative importance of e.g. genetic drift, inbreeding and laboratory adaptation on explaining these effects. For example, it is important to note that laboratory maintenance typically imposes a more ridged population age structure and raising flies in discrete generations can have significant effects on the evolution of life history traits (Roff, 1992).

Interspecific vs. intraspecific variation:

For comparative studies, it is naturally of concern if laboratory populations show characteristics that are consistently (or randomly) different from their conspecifics in the field. Additionally, when ordering from a stock center or establishing a laboratory population, it may be difficult to control how factors such as inbreeding, genetic drift, or laboratory adaptation drive differences from wild populations. A central question then becomes, how sensitive are interspecific comparisons to these intraspecific differences? By, partitioning the variance in trait values between species differences and population differences we find species to be the strongest predictor with the greatest relative importance score in all of the traits we measured (Table 2). However, we note that our study suggests variation between species starts to decrease under laboratory maintenance. Importantly, as discussed above, our analyses also demonstrate that this phenomenon does not eclipse the interspecific variation for use in comparative studies.

Conclusions:

The ability to extrapolate large-scale patterns in comparative animal ecology, physiology and evolution hinges on the ability to rely on the data obtained from animals maintained in the laboratory as well as from populations recently collected in the field. While concerns have been raised about using laboratory maintained populations (Gibbs, 1999; Harshman & Hoffmann, 2000; Terblanche & Chown, 2007), a survey of the literature failed to reveal a consistent directional response to laboratory conditions. To test this further, we generated directly comparable data of field and laboratory populations of 9 *Drosophila* species to evaluate the importance of laboratory maintenance on macro-physiological and macro-ecological

correlations. Despite differences between field and laboratory populations in specific traits, we could not demonstrate a uniform response to laboratory maintenance across 12 traits. Additionally, we show that the interspecific differences explain the majority of trait variance in all traits. Because interspecific variance is generally larger than intraspecific variance we also find that physiological and ecological trait correlations are generally similar when using data from laboratory or field populations, respectively. Together these observations validate the use of laboratory maintained populations in comparative studies. This finding is important not only for insect ecophysiology, but for comparative biology in general because it validates the use of large comparative collections of data even if these rely on research organisms that have been maintained in the laboratory for considerable time.

Data Accessibility:

Data deposited in the Dryad repository: <http://doi.org/10.5061/dryad.585t4>

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Figure Legends:

Figure 1: Summary of relevant studies comparing field and laboratory populations. There is a lack of general response to laboratory maintenance in all 28 studies identified including seven orders of invertebrates (diptera, lepidoptera, arachnid, cladocera, calanoida, acariformes, and hemiptera) (A). This is also true when the results are limited to just *Drosophila* studies (see Table S2) (B).

Figure 2: Correlation coefficients for all field (x-axes) and laboratory (y-axes) populations. Trait types are categorized into: tolerance traits, body composition, and life history. These are indicated by color and shape in the figure legend. Each graph shows both the linear regression (solid line) and the line of unity (dashed line). The correlation coefficients for all 12 traits for all species (A) show that time in the laboratory may dampen, but not negate, the relationship between traits. The high correlation between traits and environmental variables (B) demonstrates that laboratory maintenance does not erode this association.

Figure 3: Regression of the mean and standard error for each of the tolerance traits for all field (x- axes) and laboratory (y-axes) populations. Species are denoted by a combination of color and symbol type. A significant difference between field and laboratory populations as determined by Kruskal-Wallis test is noted by bold type-face and color in the species legend of each panel (blue is a decrease in the laboratory compared to the field and red is the opposite). Each graph shows both the linear regression (solid line) and the line of unity (dashed line). CT_{min} (A) and CT_{max} (B) show tight linear correlations between the field and the laboratory populations. Desiccation (C) shows a great deal of variation such that laboratory populations can survive significantly longer than field populations as seen in species such as *D. subobscura* or significantly shorter as seen in *D. melanogaster* and *D. immigrans*. Starvation (D) has a slope that is close to 1 with field populations surviving longer than laboratory populations in a number of species such as *D. sulfurigaster* and *D. rufa* but with the notable exception of *D. birchii*.

Figure 4: Regression of the mean and standard error for each of the body composition traits for all field (x-axes) and laboratory (y-axes) populations. Species is denoted by a combination of color and symbol type. A significant difference as determined by Kruskal-Wallis test is noted by bold type-face and color (blue is a decrease in the laboratory compared to the field and red is the

opposite). Each graph shows both the linear regression (solid line) and the line of unity (dashed line). Size as determined by dry weight (A) shows tight linear correlations between the field and the laboratory populations. Lipid composition (B) shows greater variation in the laboratory population.

Figure 5: Regression of the mean and standard error for each of the life history traits for all field (x-axes) and laboratory (y-axes) populations. Species is denoted by a combination of color and symbol type. A significant difference as determined by Kruskal-Wallis test is noted by bold type-face and color (blue is a decrease in the laboratory compared to the field and red is the opposite). Each graph shows both the linear regression (solid line) and the line of unity (dashed line). Fecundity (A) shows a great deal of variation such that laboratory populations can have reduced reproductive output compared to field populations as seen in species such as *D. subobscura* and *D. simulans* or significantly increased as seen in *D. melanogaster*. Development time (B) shows tight linear correlations between the field and the laboratory populations. Longevity (C) shows a great deal of variation in median survival time with laboratory population generally living longer than field populations. Resting metabolic rate at 20°C (D) and the related Q₁₀ (E) show a great deal of variation while maintaining a significant correlation between laboratory and field populations. The proportional activity (F) is highly variable but with field populations of *D. melanogaster* and *D. hydei* moving significantly more than their laboratory conspecifics.

Table 1: Information on establishment and maintenance of species pairs used in this study. Numbers of generations of maintenance was estimated from generation time and length of time in the laboratory. More comprehensive information can be found in Table S2.

Table 2: For each trait, we analyzed the trait value in a linear model with species, population, and the interaction as predictor variables. We also included a calculation of relative importance values for each predictor. Species explains a mean of 88.3% of the variance with a minimum of 77.6% and population (field or lab) only explains a mean of 1.6% of the variance with a maximum of 8.67.