BIOLOGY LETTERS

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Cite this article: Teague C, Youngblood JP, Ragan K, Angilletta Jr MJ, VandenBrooks JM. 2017 A positive genetic correlation between hypoxia tolerance and heat tolerance supports a controversial theory of heat stress. *Biol. Lett.* **13**: 20170309. http://dx.doi.org/10.1098/rsbl.2017.0309

Received: 19 May 2017
Accepted: 18 October 2017

Subject Areas:
evolution, ecology

Keywords:
Drosophila, hypoxia, oxygen, performance, temperature, tolerance

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Physiology

A positive genetic correlation between hypoxia tolerance and heat tolerance supports a controversial theory of heat stress

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We used quantitative genetics to test a controversial theory of heat stress, in which animals overheat when the demand for oxygen exceeds the supply. This theory, referred to as oxygen- and capacity-limited thermal tolerance, predicts a positive genetic correlation between hypoxia tolerance and heat tolerance. We demonstrate the first genetic correlation of this kind in a model organism, *Drosophila melanogaster*. Genotypes more likely to fly under hypoxic stress (12% O_2) were also more likely to fly under heat stress (39°C). This finding prompts new questions about mechanisms and limits of adaptation to heat stress.

1. Introduction

Biologists hold competing ideas about heat stress, but overheating likely depends on processes that differ among life stages or species. Some biologists believe that death precipitates from proteins and membranes losing their integrity as kinetic energy increases, disrupting ionic gradients or reducing enzymatic activity [1]. Others argue that a systemic failure to deliver oxygen during heating accelerates the loss of cellular functions, because an organism cannot meet its demand for energy through anaerobic metabolism alone [2–5]. This alternative view means that oxygen limits function before kinetic energy becomes deleterious. Most evidence that oxygen limits heat tolerance comes from studies of animals living in water, such as marine fish [6], aquatic insects [7], or developing embryos [8,9]. By contrast, most terrestrial animals are insects, whose tracheal systems seem to deliver sufficient oxygen during heating, especially under ecological levels of hypoxia [10]. Yet, most experiments have focused on animals at rest. Therefore, more insight might come from studies of heat tolerance during metabolically expensive activities [11].

Quantitative genetics [12,13] can establish a mechanistic link between oxygen delivery and heat tolerance during activity. If oxygen supply limits thermal tolerance, a positive genetic correlation should exist between performance during hypoxia and performance during heating [14]. Such a correlation would manifest as phenotypic covariation among genotypes [15], such that genotypes delivering oxygen better under hypoxia also tolerate heat better under normoxia. By controlling for environmental factors, one can attribute the phenotypic covariance measured among genotypes to genetic factors. Thus, the phenotypic correlation among clonal lines reflects a genetic correlation. Since a genetic correlation reflects interactions between biochemical pathways that determine phenotypes [16], we can test the theory more directly by quantifying this genetic correlation [17–19].

Table 1. A model relating the probability of flight at 39°C to the probability of flight at hypoxia ($P_{hypoxia}$) was far more likely to fit the data than a model that also included the probability of flight under no stress ($P_{unstressed}$), or a model with no independent variables (null). All models included an intercept and an error term. For each model, we report the number parameters (*k*), the log likelihood, the Akaike information criterion (AIC) and the Akaike weight.

model	*k*	log likelihood	AIC	ΔAIC	*w*
$P_{hypoxia}$	3	−99.6	206.5	0.00	0.74
$P_{hypoxia} + P_{unstressed}$	4	−99.6	209.4	2.91	0.17
null	2	−103.9	212.4	5.94	0.04
$P_{hypoxia} + P_{unstressed}$ + interaction	5	−99.6	212.6	6.13	0.03
$P_{unstressed}$	3	−103.4	213.9	7.46	0.02

We quantified the genetic correlation between flight performance under hypoxic stress and flight performance under heat stress in *Drosophila melanogaster*, a species frequently used to explore genetic variances and covariances [20–22]. We compared isofemale lines from the *Drosophila* Genetic Reference Panel [23], which have been used to estimate genetic parameters for behavioural and physiological traits [24–26]. Because each line consists of a distinct genotype, a phenotypic correlation between traits reflects an additive genetic correlation [18]. Here, we report a genetic correlation between the ability to fly during hypoxic stress and the ability to fly during heat stress—the first genetic evidence that heat tolerance depends on the capacity to deliver oxygen.

2. Material and methods

Flies from 24 isofemale lines were raised in vials of standard medium (Bloomington Stock Center, Indiana University) inside a programmable incubator (Percival Scientific, Perry, IA, USA) at 25°C, 50% humidity, and a 12L : 12D photoperiod. The 24 lines were chosen to encompass a wide range of genetic variation [23] (see Dryad for the identities of these lines [27]). Each generation, we controlled the density of each line by allowing two females to lay eggs in a new vial for 48 h. After two generations, female metamorphs were anaesthetized with CO_2 and transferred to fresh vials. At 6–8 days of age, these flies were anaesthetized and transferred to separate vials. The next day, each fly was transferred without anaesthesia to an empty vial for the flight test.

We tested the flight performance of 20 flies from each line under three conditions: heat stress (39°C and 21% oxygen); hypoxic stress (25°C and 12% oxygen); and no stress (25°C and 21% oxygen). These levels of heat and hypoxia reduced flight performance in a previous study [11]. We used a custom flight chamber constructed from clear acrylic (25.4 × 25.4 × 25.4 cm). Air temperature was controlled by placing the chamber in a walk-in incubator (EGC, Chagrin Falls, Ohio). The atmosphere in the chamber was regulated within 0.5% by a ROXY-1 Universal Controller (Sable Systems, Las Vegas, NV, USA). Flies entered the chamber through a circular hole in the top (2.5 cm in diameter), sealed by a movable plate. For each test, a vial with a fly was inverted onto the plate. Holes in the plate enabled the oxygen concentration of the vial to equilibrate with that of the chamber (less than 4 min). While the fly was on the wall of the vial, a researcher slid the plate aside and tapped the fly into the chamber. Flies either fell uncontrollably or flew to a surface. A fall was scored when a fly landed on the floor within 10 cm of the point below the vial. In previous studies, this index of flight performance varied predictably over acute, developmental and evolutionary time scales [11,28], suggesting ecological relevance. Because measurements were made over several days, we tested flies from each isofemale line in a random order on each day. Each fly was tested at a single environmental condition. Flight under each condition was tested on alternating days.

We used model averaging [29] to relate the probability of flight during heat stress to the probability of flight during hypoxic stress and the probability of flight under no stress. The MuMIn library [30] of the R Statistical Package, v. 3.1.1 [31] was used to fit all possible linear models to the data. Then, we calculated the likelihood that each model best describes the data (table 1). Finally, the expected probability of flight under heat stress was computed from the weighted average values of parameters.

3. Results and discussion

We found a positive genetic correlation between performance under hypoxic stress and performance under heat stress (figure 1). A model relating these variables ($\beta = 0.99$, s.e. = 0.35, $p = 0.01$) was 4 times more likely than any other model and 18 times more likely than the null model (table 1). In other words, genotypes that flew more often at 25°C and hypoxia also flew more often at 39°C and normoxia. The likelihood that performance under heat stress depended on performance under hypoxic stress was 94%, based on the Akaike weights of models including this variable (table 1). Far more variation in flight performance under heat stress was related to flight performance under hypoxia ($r^2 = 0.30$; figure 1*a*) than was related to flight performance under no stress ($r^2 = 0.04$; figure 1*b*). Thus, the genetic correlation between hypoxia tolerance and heat tolerance could be as high as 30% [18], which lies within or above the range of genetic correlations for other traits in *D. melanogaster* [20,32].

Although the genetic correlation between hypoxia tolerance and heat tolerance might have resulted from physical linkage between unrelated alleles, this correlation more likely points to alleles conferring a capacity to fly under both conditions. Genetic variation in the delivery or consumption of oxygen during flight could link these performances. Insects rely almost completely on aerobic metabolism during flight, which in turn depends on sufficient delivery of oxygen to mitochondria [33,34]. Insects adapted to low oxygen might have larger wings, smaller bodies, more elaborate tracheal systems, efficient muscle fibres, higher mitochondrial densities, or specialized metabolism [35,36]. These same properties could account for variation in hypoxia tolerance within populations. Additionally, tolerant genotypes may ventilate their tracheoles faster, for example, by opening spiracles longer and more frequently [37]. Finally, these genotypes may have more efficient ion pumps to maintain gradients with less ATP [38], which also enables some genotypes to tolerate cold better than others [39,40]. Further studies are needed to

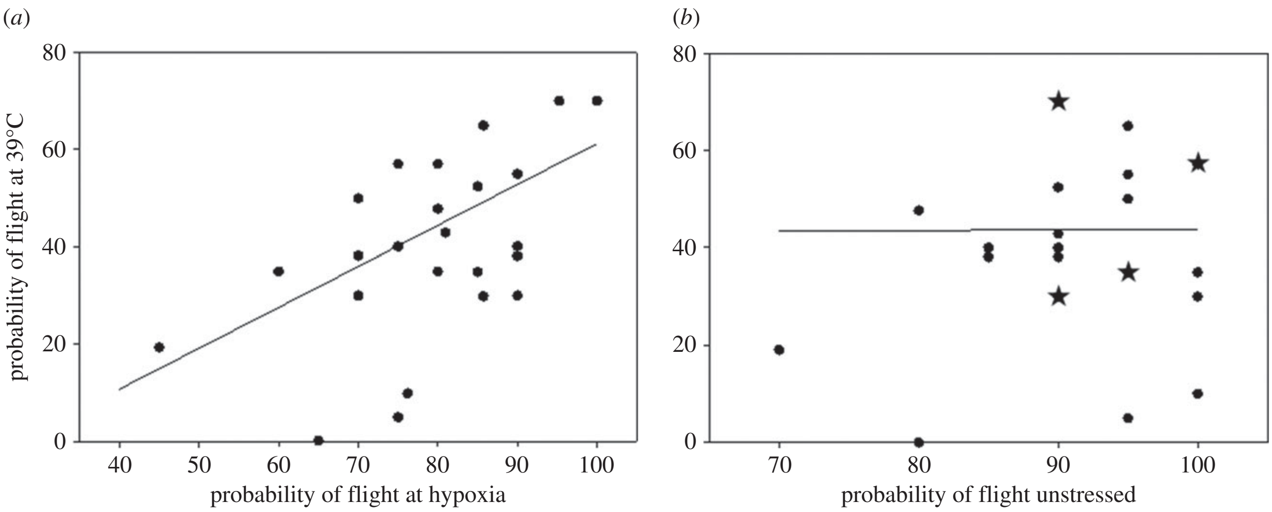

Figure 1. The probability of flight at 39°C and normoxia was genetically correlated with the probability of flight at 25°C and hypoxia (*a*) but not with the probability of flight under no stress (*b*). Each point represents the probabilities of flight for an isofemale line; stars denote identical values for two isofemale lines. Lines represent the relationships determined by multimodel averaging.

confirm which, if any, of these mechanisms underlie the genetic correlation reported here.

Researchers should reconsider the extent to which oxygen supply limits the heat tolerance of terrestrial animals [41,42]. Despite doubts about the generality of oxygen-limited heat tolerance [10,11,43], animals could succumb to over-heating from a lack of oxygen when engaged in metabolically demanding activities such as flight. This new evidence builds on the recent discovery that oxygen supply limits the heat tolerance of embryonic animals [9]. Thermal tolerance relies on mechanisms that regulate energy, water and ions, each of which likely dominates in a certain environmental context or life stage. Even if oxygen limitation fails to explain heat stress in all circumstances, this model might become more useful when considering the life stages and aerobic activities most susceptible to hypoxia [2].

Data accessibility. Data available from Dryad: https://doi.org/10.5061/dryad.k95p5 [27].

Authors' contributions. C.T., J.M.V. and M.J.A. designed the study. C.T., J.P.Y. and K.R. collected data. J.P.Y. analysed data. C.T. and J.P.Y. drafted the manuscript. All revised the manuscript, approved the final version, and agreed to account for its content.

Competing interests. We have no competing interests.

Funding. Research was supported by ASU and MWU.

Acknowledgements. We thank Gerald Call for isofemale lines and Marcin Czarnoleski for valuable discussion.

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