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A SIMPLE METHOD FOR OBSERVING AND MEASURING HEART RATES IN LIVE ADULT MONARCHS (*DANAUS PLEXIPPUS*) AND OTHER LARGE BUTTERFLIES

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ABSTRACT. Heart rates of insects reflect the current level of activity and stress individuals are experiencing, and therefore this information can be useful from a research perspective. In Lepidoptera, as with most insects, the 'heart' consists of a transverse longitudinal tube that runs along the abdomen, just under the abdominal tergites, which pumps or contracts rhythmically to distribute hemolymph. Here, we describe a simple method we developed to observe heart contractions in adult monarch butterflies, *Danaus plexippus*. The procedure involves stabilizing the live specimen in a pre-prepared plastic bag, while positioning the abdomen in such a way so that the beating heart can be seen (with magnification) through the intersegmental membranes. With this procedure, no harm comes to the specimen, making the technique useful in both lab and field studies. The technique also requires little equipment, except for a dissecting microscope (or other magnifier). Moreover, the procedure should be useful for monitoring other similarly-sized Lepidoptera, and we confirmed this with a *Papilio glaucus* specimen. Using this method on 10 male and 10 female monarch specimens from captive-reared stock, we found the resting heart rate was 63 beats/min on average (range: 35–86). This information will be useful for comparative purposes, or as a reference point for future studies of monarch physiology.

Additional key words: heart rate, monarch butterfly, *Danaus plexippus*, physiology

There is a long history of scientific investigation into the physiology of the insect heart, with observations from early scientists dating as far back as the 19th century (Newport 1837). The insect heart consists of a transverse tube (the 'dorsal vessel') that runs along the dorsal section of the abdomen, which pumps or contracts rhythmically to distribute hemolymph from the anterior to the posterior parts of the body. Over the years there have been various methods employed to monitor these heart contractions, including using electro-mechanical transducers (Senff 1971), infra-red sensors (Kuusik et al. 2001), and more recently, electrical resistance measurements (Wasserthal 2012). One of the earliest and simplest procedures came from Newport (1837), who scraped the upper abdominal scales off of a hawk moth (*Sphinx ligustri*) in order to view the pulsations of the dorsal vessel. He noted when the specimen was at rest, the heart rate was 42–49 beats/min, and after the moth had "flown around his sitting-room" for several minutes it was 127–139 beats/min. Other studies where insect heart rates have been monitored have been extensively reviewed by Jones (1974), and from this review it is clear that there is a wide range of heart rates among insect orders and across species within orders. Also notable from this review is that within the Lepidoptera, nearly all research efforts have focused on either larval or pupal stages.

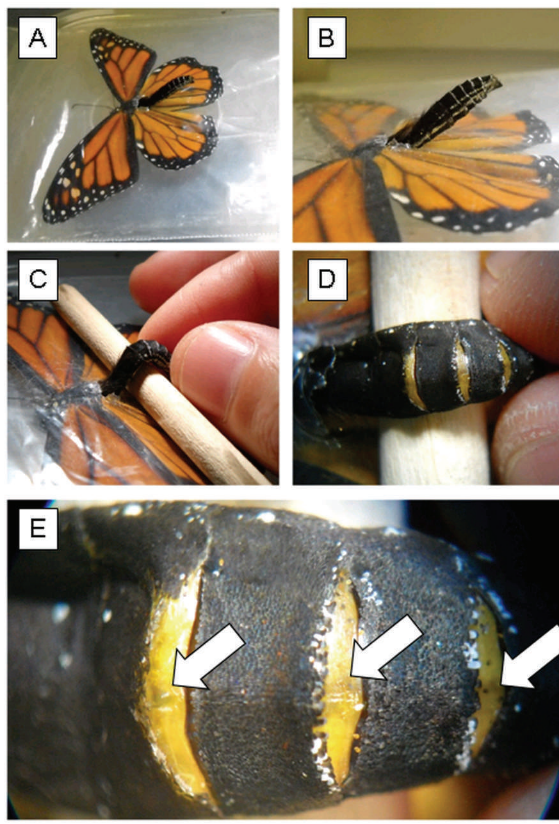

FIG. 1. Visual depiction of the heart observation of monarch butterflies, *Danaus plexippus*. The live monarch is placed in a clear plastic pouch with wings open and abdomen protruding from a small hole (**A, B**). The abdomen is gently pressed down over a round object (a pencil, or probe handle, as in **C**), which stretches the intersegmental membranes (**D**). The dorsal vessel (heart) can be visualized under these membranes (**E**) under a low-power dissection scope.

Here, we describe a simple method we developed to observe heart contractions in adult monarch butterflies, *Danaus plexippus*. The procedure allows live monarchs to be monitored without harm (and without scraping off scales), requires minimal equipment (except for a low-power dissection scope), and it requires no formal expertise except in safe handling of butterflies. Note that while we focused on the monarch, this procedure would be equally-suited for use on any similarly-sized or larger butterfly species, such as most swallowtails (genus *Papilio*).

Procedure description. To begin, the live monarch (or other butterfly) is placed in a clear plastic ziplock bag, which has a small (1cm diameter) hole cut into one side. The butterfly is positioned in the bag with its wings opened horizontally, then moved so that its abdomen extrudes through the hole (Fig. 1A,B). The sides of the bag are then weighed down so that the butterfly remains immobile, which frees up the observer's hands. Next, a round object such as a pencil or a probe handle is placed under the abdomen (Fig. 1C), and with a free hand, the observer gently presses the abdomen over the object (Fig. 1D). In this position, the intersegmental membranes become stretched (Fig. 1E), which allows the dorsal vessel (i.e. the heart) to be observed with a low-power dissection microscope, or other visual aid, such as a visor magnifier. With practice, butterflies can be held in this position for several minutes, while the observer watches and counts the pulses of the heart (although a 1-minute interval is sufficient).

Monarch heart rates. Using this method we evaluated the resting heart rates of 20 live monarchs (10 males, 10 females) that had been reared in captivity as part of other projects during summer 2015 at the University of Georgia. These butterflies were progeny of several generations of captive stock (originating from wild-caught adults from Texas and Georgia), and had been fed cuttings of greenhouse-grown *Ascelpias*

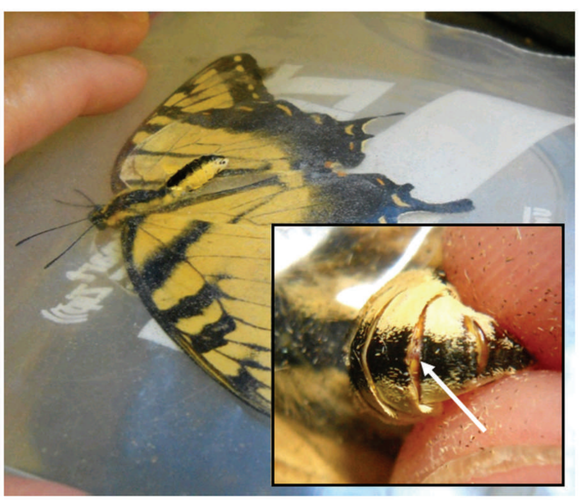

FIG. 2. The technique was evaluated on a *Papilio glaucus* specimen; the same procedure was followed, including positioning of the specimen in the bag, and stretching the intersegmental membranes (inset) to see the dorsal vessel contracting (arrow).

TABLE 1. Summary of heartbeat measurements for male and female monarch butterflies in this project. All heartbeat values are expressed as the number of beats/min. There was no significant difference between sexes in average heart rate.

Sex	N	Mean	SD	Min	Max
Females	10	65.9	17.8	35	85
Males	10	60.5	16.1	37	86
Both Groups	20	63.2	16.7	35	86

incarnata as larvae. As adults, they had been stored in glassine envelopes in an incubator set to 14°C for one month, and removed once a week for feeding, which was a 1:4 mixture of honey-water. For the heartbeat readings (conducted the week of Sept 7–14, 2015), we removed the monarchs from the incubator (but not from their envelopes) 30min prior to assessment. The temperature of the room was constant at 22°C. The average resting heart rate of the 20 monarchs was 63.2 beats/min (16.7 SD), and ranged from 35 to 86 (Table 1). While female heart rates were slightly higher than that of males, the difference was not significant (Student's t-test, df=18, t=0.712, p=0.485).

As mentioned previously, the lack of comparable data (i.e. from adult, intact specimens, measured visually) from other Lepidoptera species makes it difficult to know if the data above are normal for adult monarch butterflies. Similar visual-based observations from non-manipulated mosquitoes (*Anopheles gambiae*) indicated an average rate of 82 beats/min (Glenn et al. 2010), and heart rates of the adult moth *Mamestra brassicae* were between 60–90 beats/min, measured with electrocardiographs (Queinnec & Campan 1972). Interestingly, other research from our lab has found horned passalus beetles (*Odontotaenius disjunctus*) have an average resting heart rate comparable to monarchs, averaging 63 beats/min (Davis, unpubl. data). From this collective information we surmise that the heart rate data we collected is in line with results from prior work on insects. At the very least, these values for monarchs should be of use as a baseline which future projects could add to.

The procedure outlined here should be useful for observing heartbeat of other similarly-size Lepidoptera. As a test of this, we examined a wild-caught tiger swallowtail (*Papilio glaucus*) specimen, following the same steps outline above, and had no difficulty seeing the heart contractions (Fig. 2). Other strengths of this technique are its ease of use, and the fact that it requires no advanced equipment besides a dissecting microscope, or some other magnifying tool (such as a visor magnifier). Moreover, this procedure does not injure the butterfly, and does not require removal of abdominal scales, as Newport did 178 years ago (Newport 1837). We note that removing abdominal scales actually may not be effective for observing heart contractions in all Lepidoptera; we attempted to do this with the *P. glaucus* specimen, but the abdominal tergites in this species are dark (i.e. not translucent), and the underlying dorsal vessel was not easily seen. In contrast, the inter-segmental membranes are translucent on most species (compare Fig. 1 and Fig. 2), regardless of the species' integument color.

Potential applications. Given that the procedure does not injure the butterfly, this technique could be used for a wide variety of research applications, both in the lab and in the field. For example, a defining characteristic of the monarch is its long-distance migration in eastern North America (Brower 1995, Howard & Davis 2009), and in theory this procedure would allow migrant monarchs to be captured, assessed in the field, then released to continue their migration. This information could be compared with the heart rates in other behavioral activities to quantify relative energy expenditure in each state. Other questions could be addressed relating to stress and stress susceptibility. Transient increases in heartbeat frequency are a universal response to acute stressors in vertebrates and invertebrates (Beerda et al. 1998, Rushen et al. 2001, Terkelsen et al. 2005, Papaefthimiou & Theophilidis 2011). Thus, knowing what factors cause heart rates to increase in monarchs could help to identify times or conditions under which monarchs become stressed. This may include exposure to agricultural chemicals (Pecenka & Lundgren 2015), and/or being infected with parasites (Altizer & Oberhauser 1999). While research into these issues is ongoing, so far there has been no evaluation of how much physiological stress these factors cause to individual monarchs. Because of its many potential applications, the technique described here should be useful to Lepidopterists, and moreover, it will hopefully help to reinvigorate new investigations into heart rate variations in insects.

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