# Fecundity and longevity of houseflies after space flight[1]

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*Summary.* After 7 days of space flight house flies, *Musca domestica,* exhibited similar longevity, but a reduction in reproductive output as compared to earthbound controls. This reduction was not observed in later generations. These data suggest that space flight directly alters the rate of ovarian development, but that this effect is not genetically transmitted.
*Key words.* Space flight; reproduction; longevity; insect.

The biological hazards of prolonged weightlessness during space travel are unknown. Many biological processes may require gravitational forces for normal function (i.e. the formation of morphogenic gradients essential for early embryogenesis[3]). Our ability to detect such effects has been severely limited, particularly for long-lived complex organisms; however, a few insects have been exposed to weightlessness for a substantial portion of their life cycle and may provide appropriate test models. Male *Drosophila melanogaster* aboard Cosmos-936 for 18.5 days exhibited normal development but reduced longevity compared to earthbound controls[4]. Female velvetbean caterpillars exposed to weightlessness for 7 days on the third flight of the Space Shuttle Columbia showed no clear loss of longevity or reproductive capacity after return to earth[5]. Houseflies (*Musca domestica* L.) were also carried on this same space shuttle mission and were returned to our laboratory where tests on longevity and reproductive capacity were carried out. Although there were few flies included in this flight (see below), the paucity of available information on the effects of weightlessness warranted the investigations here, where we show for the first time a reduction in reproductive potential due to space flight.

Prior to the flight of the shuttle, puparia were divided into two groups: one group was placed in a flight chamber while a second control group was placed in a parallel chamber to remain at Johnson Space Center. Pupae were aged to begin emergence of adults during the first day of flight so that late pupae and early adult development occurred in space. To control for gravitational stress during launch, the control group was subjected to $3 \times g$ for 550 sec, simulating lift-off. Both groups were supplied continuously with a dilute sugar solution consisting of 12.1% sucrose, 8.0% glucose and 0.9% fructose.

1 day after flight all flies were returned to our laboratory where they were individually caged at 27 °C and 50% relative humidity in 325-ml cups that had the bottoms replaced with netting and inverted over a petri dish. A total of seven female and three male flies of an initial 12 pupae emerged in space, while seven females and five males of an initial 12 pupae emerged in the control. Flies were fed daily with a dilute solution of evaporated milk, and at 2–4-day intervals females were provided with CSMA larval medium for oviposition[6]. All females were checked daily for egg laying (although females only laid eggs when CSMA medium was provided). Egg batches were transferred to rearing jars containing CSMA larval medium where egg hatching after 48 h and subsequent adult emergence was recorded. Thus we were able to obtain life history information (longevity and reproductive history) of these flies from both the shuttle mission and the simulator controls (table 1).

Flies emerged from 22 of the 24 pupae, with the two pupae not emerging from the shuttle sample of 12 (not significantly different at $p > 0.05$). Of the flies emerging, the average life span of females was 34.2 days and 34.4 days for the space shuttle and simulator flies, respectively, while that for males was 26.0 days and 17.6 days, respectively. Clearly there was no significant reduction in longevity of the flies exposed to weightlessness. On the other hand, the percentage of females laying eggs at the first opportunity they were provided with CSMA medium was significantly reduced in the space shuttle (28.6%) versus the simulator flies (85.7%). These space shuttle females continued to lay egg batches at a slower rate throughout their lives: both the total number of egg clutches and the total lifetime fecundity were less in the space shuttle females (although the number of eggs per clutch was nearly identical in the two groups). The number of eggs laid is intimately related to fly size; however, the average length of wings, as an indicator of female size[7], was identical in the two groups. This was not a cause of the reduced fecundity. The percent of eggs that hatched and the subsequent survival to adulthood of these hatched larvae were not significantly different between the two groups.

To find out if these effects continued during subsequent generations single female cultures were set-up in the same manner as before, from shuttle and simulator populations derived from the original flies and maintained in the laboratory for 25 generations. The percent of females ovipositing at the first opportunity, the percent of clutches laid per opportunity, the number of eggs per clutch, the number of clutches, the total number of eggs laid and the percent of eggs hatching were all nearly identical (and

Table 1. Average longevity and reproduction of flies ( ± SE) exposed to weightlessness of space compared with earth controls

| Measure | Shuttle | Simulator |
|---|---|---|
| Pupal emergence | 83.3 | 100.00 |
| Adult longevity (in days) | | |
| Males | 26.0 ± 2.0 | 17.6 ± 2.6 |
| Females | 34.4 ± 2.6 | 34.2 ± 4.3 |
| % females ovipositing at first opportunity | 28.6* | 85.7 |
| % females ovipositing per opportunity | 59.1* | 89.7 |
| Eggs/clutch | 92.9 ± 15.6 | 98.6 ± 4.1 |
| Total egg clutches per female | 4.5 ± 1.3 | 6.2 ± 0.7 |
| Total number of lifetime eggs laid per female | 390 ± 99.9 | 532 ± 50.3 |
| % eggs hatching | 90.1 ± 4.6 | 98.6 ± 0.4 |
| % larval survival | 75.4 ± 6.0 | 79.8 ± 6.0 |

p-values were determined by a one-tailed Mann-Whitney U test, except for the comparison of pupal survival and percentages of females ovipositing at the first opportunity which was tested by Fishers Exact Test[8]. An asterisk represents a significant reduction in preformance of shuttle flies at $p < 0.05$.

Table 2. Average reproduction ( ± SE) in housefly females at generation 25 from laboratory populations derived from the original shuttle and simulator flies

| Measure | Shuttle | Simulator |
|---|---|---|
| % females ovipositing at first opportunity | 84.6 | 83.3 |
| % females ovipositing per opportunity | 79.0 ± 4.7 | 75.0 ± 5.6 |
| No. eggs/clutch | 90.6 ± 3.8 | 88.5 ± 3.8 |
| Total egg clutches per female | 5.3 ± 0.6 | 5.3 ± 0.5 |
| Total number of lifetime eggs laid per female | 482 ± 49.6 | 472 ± 32.8 |
| % eggs hatching | 95.8 ± 1.6 | 96.2 ± 1.0 |

not significantly different) between these females (table 2). Thus there were no apparent residual effects of the space experience in these later generations.

These data suggest that there were some immediate affects of weightlessness on female houseflies emerging in space. These effects seem to be limited to the rate of egg development and did not affect either the number of eggs per each egg batch or the survival of offspring from these eggs. These differences were not owing to differences in size of female flies. Also, these flies were not provided an appropriate protein diet during the flight necessary for egg development[9] and thus egg development occurred after the flight at normal gravity conditions. Hence, there was a presumed direct affect of weightlessness on ovarian development that altered the rate of subsequent oviposition. If these effects are indeed due to the shuttle experience per se, they represent the first documentation of reduced reproductive ability in a complex organism exposed to space travel. These limited data at least suggest that additional investigation is warranted.

1 Acknowledgment. We thank Todd E. Nelson, student participant in the Space Shuttle Student Involvement Project, John T. Jackson, NASA/Johnson Space Center, and James R. Peterson, Honeywell, Inc. for providing us with the flies and information related to the in-flight experiments.
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0014-4754/85/091191-02$1.50 + 0.20/0


## Immunological systematics of the extinct quagga (Equidae)

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*Summary.* It has been debated whether the extinct quagga was a distinct fourth species of African zebra or whether it was merely the southern variant of the Plains zebra *(Equus burchelli)*. Using a radioimmunoassay (RIA) technique, we have shown that proteins remaining in quagga skins from museums are much more similar to serum proteins of the Plains zebra than to those of the other two extant zebras.
*Key words.* Quagga; zebra; horses, equids, radioimmunoassay; molecular evolution; proteins.

The quagga *(Equus quagga quagga)* was once the most numerous zebra-like animal found in southern Africa. Like the plains bison of North America, this large mammal was found in herds of inestimable size, but human activities destroyed their habitat, and human predation for skins and meat decimated their numbers[1]. The last quagga died alone in her stall at the Amsterdam zoo on 12 August 1883[2]. Only 23 quagga skins are now known to exist in museums[3].

Some controversy surrounds quagga systematics. The dominant hypotheses suggest that it represented either the fourth species of African zebra or, alternatively, that the clinal variation in striping pattern of the Plains zebra, *Equus burchelli,* included as its southernmost variant the quagga[4-8]. Using radioimmunoassay (RIA), we have shown that immunologically quagga skin is very similar to skin and serum of the Plains zebra.

Previous systematic analyses of zebras have depended largely on ranges and striping patterns. The quagga existed in Cape Province, south of the Orange River, at the southern extension of the range of Plains zebras, which in turn were subdivided by various systematists into between six and more than 30 races. The quagga was first described by Gmelin in 1788 as a distinct species[9]. The sportsman W.C. Harris also considered it as a distinct species whose range overlapped that of the Plains zebra in the Orange Free State[4]. Our present confusion is compounded by the fact that the terms quagga and zebra were often used interchangeably, and only a single quagga – the one at the London zoo – was ever photographed in life[2]. More recently, E.C. Mungal[6] argued that despite its obviously close similarity in striping pattern with the Plains zebra, the quagga's occupancy of the karroo habitat, distinct from the grassland habitat of Plains zebras, supports the impression of contemporary observers that this form was a separate species.

Conversely, the clinal variation in striping patterns of the Plains zebras thoughout their range from East Africa to the Transvaal has led other authors to consider the quagga as the extreme southern variant[3,7,8]. The two other extant species of zebras are the Mountain zebra *(Equus zebra)*, whose range includes South Africa and Namibia, and the Grevy's zebra *(Equus grevyi)*, whose range is East Africa. Because the quagga is more similar in range and pelage to the Plains zebra than to the others, the major systematic issue centers on the question whether the quagga is or is not a distinct species.

All extant species of zebras display unique karyotypes[10]. Unfortunately, we do not have the technology for doing karyotypes of extinct animals. In recent years, however, it has proved possible to extend molecular systematic studies to extinct species, notably through use of an RIA method[11].

*Materials and methods.* Recently specimens of several of the existing quagga skins have been made available to us for molecular analysis. We also obtained skin about 70 years old from a Plains zebra, the type specimen of *E. burchelli paucistriatus,* now synonymized with *E. burchelli burchelli.* Approximately 1 g skin was finely ground and extracted with EDTA 0.2 M. This solution was used as antigen in a solid phase RIA[11]. Additional antigens consisted of the sera of the three extant zebras, the two African ass subspecies *(E. asinus f. asinus* and *E. asinus somaliensis)*, the two Asiatic wild ass subspecies *(E. hemionus onager* and *E. hemionus kulan)* and the domestic and Przewalski's horses *(E. caballus* and *E. przewalskii)*. Antisera to these nine sera were raised in rabbits by spaced injections[11]. Direct cross-reactions