Grasshoppers in research and education: methods for maintenance and production

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Insects used in research have traditionally been housed and cared for in the investigator's laboratory. Centralized colony maintenance may be advantageous, but presents unique challenges to animal care staff members, who are more familiar with vertebrate research animals. To fill this potential knowledge gap, the authors share the procedures they have developed at Arizona State University for the housing, husbandry, and breeding of grasshoppers used in research and teaching.

Vertebrates comprise the vast majority of species for which an institution's animal care facility (ACF) bears responsibility. Occasionally, though, species without backbones are more suited to a particular investigation or instruction. Sea urchins, fruit flies, bees, and locusts have long been the domain of geneticists, developmental biologists, ecologists, and students of social organization. The horseshoe crab also has a distinguished history in these realms[1].

Information gained from the study of insects as laboratory animals has numerous implications for humans and other mammals. The role of various insects as vectors of disease, for instance, has long been recognized. Moreover, the Malphighian tubules of several insects are excellent models for the study of renal function and transport[2]. Locusts have even been the subject of a recently published laboratory study characterizing mass-migratory behaviors that allow mathematical modeling, as well as application to other species that behave collectively[3].

Increasingly sophisticated technologies and instrumentation miniaturization have made some species very appropriate for defining and demonstrating basic physiologic and reproductive processes. Specifically, grasshoppers have been used to study muscle physiology, cell biology, nutrition, olfaction, and other neurosensory phenomena. Teaching labs have been built around several of these areas[4–7]. Sequence data from the genomes of invertebrate species suggest that more basic proteomic and metabolomic studies will be forthcoming. A new genomic database[8] makes it likely that grasshoppers may receive more interest and attention. As grasshoppers are recognized as increasingly valuable research models, more central animal facilities will seek information regarding their husbandry; concrete resources that draw upon the husbandry experience of researchers that have begun maintaining grasshopper colonies will be needed.

Research at Arizona State University by one of us (J.H.) has required maintenance of colonies of several species of grasshoppers beginning in 1991. These studies have been aimed at achieving a better understanding of metabolic, respiratory, and acid:base homeostatic mechanisms[9–12]. Grasshoppers are models well-suited to the study of many areas important in higher-order organisms, especially work with conduction velocities and fiber recruitment.

Unfortunately, grasshoppers are not readily available commercially. For investigators or teaching labs to have access to animals other than preserved specimens necessitates the maintenance of on-site colonies. As a starting point for our own grasshopper husbandry efforts, we, like others cultivating unusual species, relied upon sketchy notes, references to reports or methods in older texts[13,14], and anecdotal accounts from care technicians who had some experience with the animals in other laboratories. From there, we developed the standard procedures described below. Our husbandry efforts thus far have focused on two species: *Schistocerca americana,* the American locust (which we have cultured for 39 generations) and *Melanoplus femurrubrum*, the red-legged grasshopper (which we have raised for 19 generations). *S. americana* must be kept under permits from the Arizona Department of Agriculture and the

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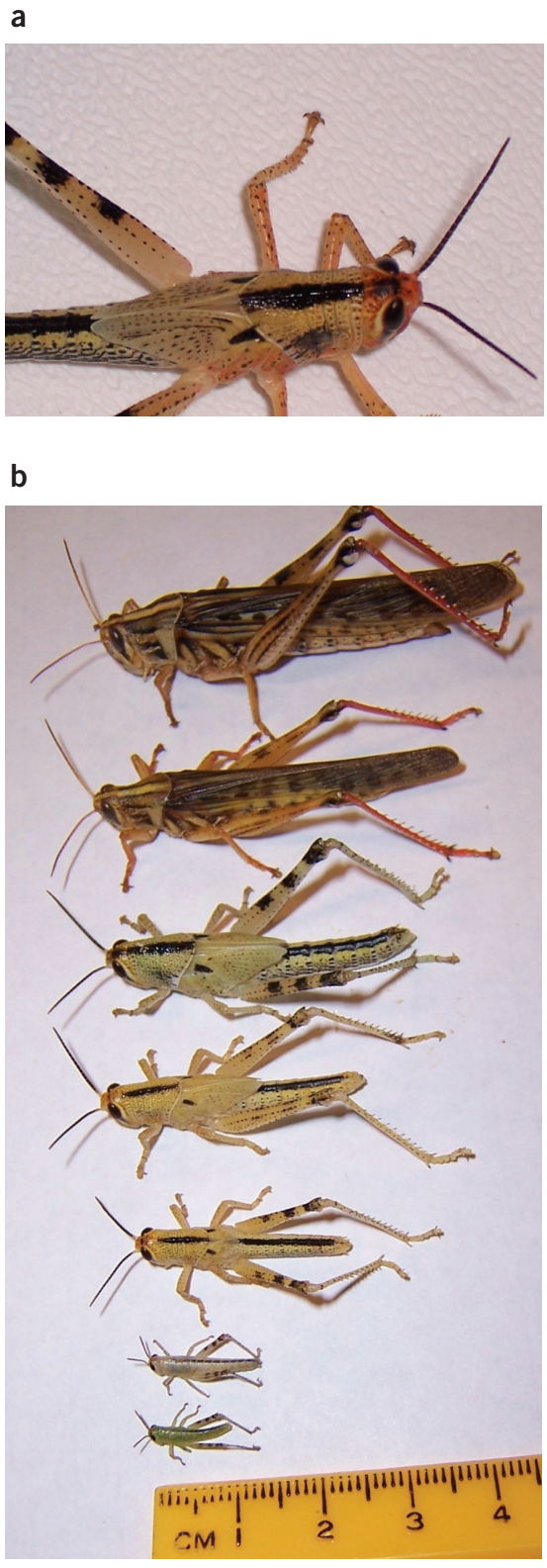

FIGURE 1 | The life cycle of grasshoppers involves six instars (molts). (**a**) Juvenile grasshoppers have small wings. (**b**) Through several molts, juvenile grasshoppers mature into adult, full-winged grasshoppers (seen at top).

US Department of Agriculture because they are plant-eating insects maintained outside their native area.

HUSBANDRY

Facility

A grasshopper husbandry room must be equipped to prevent escape. To this end, our grasshoppers are maintained in an interior room within a facility, access to which is limited to authorized users and staff members by card-reader electric/magnetic locks; all staff are trained in grasshopper escape prevention. The heating and cooling capability of the room is adequate to ensure an ambient temperature range that fluctuates between 68 °F and 95 °F (20–38 °C). Studies suggest that the optimal daytime temperature for most grasshoppers is between 86 °F and 104 °F (30–40 °C) (ref. 15). Normally, their temperature will not fall below ambient. However, if a 75-W or greater light bulb is placed near or within the cage, the grasshoppers will thermoregulate by adjusting their distance from the bulb, even if the room is kept at 25 °C. These insects are tolerant of cool (but not freezing) nighttime temperatures. All rooms in which grasshoppers are housed have at least 10–15 air changes/hour with unrecirculated fresh air.

Life cycle

The life cycle of the grasshopper begins with the development of a fertilized egg. It takes about three weeks for eggs to hatch. This is followed by a regular progression of larval intermediate stages involving sequential molts, called instars, during which they grow and mature. Grasshoppers usually go through six larval instars, each lasting about one week, for a total of about six weeks from hatching to maturity. At each instar they shed their cuticle (exoskeleton). Beginning with the fifth instar, short wing-like structures are visible on their backs (**Fig. 1a**); once they have full wings, the grasshoppers are considered adults (**Fig. 1b**). The newly adult grasshoppers have soft, pink cuticles for the first few days, which then darken in color and harden after about a week. Once the grasshoppers have changed color, they are reproductively mature and will normally begin to lay eggs. Adults live ~5–8 weeks. In general, adults may be euthanized after they are six weeks old to maintain the vigor of the colony. The size of the colony should be determined by the number of animals necessary for use.

Housing

Adult grasshoppers are housed in groups of 100–200 individuals. Cages measure 18 (width) × 36 (height) × 16.5 (depth) inches and are constructed of ¼-inch diameter wire mesh (**Fig. 2a**). The front of the cage has a top and a bottom opening for accessing grasshoppers. The top opening is 8 × 8 inches, while the bottom opening is 12 × 12 inches. Each opening is covered by an appropriately sized piece of ¼-inch diameter wire mesh

attached to the cage on one side using metal ties and on the other side using a metal spring with a clip attachment. The inside of the cage has a vertically hung 8-inch-wide piece of ¼-inch diameter wire mesh for the grasshoppers to climb on (**Fig. 2b**). The piece of mesh is attached to the top and bottom of the cage using metal ties. The grasshoppers can use the vertical mesh both to thermoregulate (see above) and to facilitate molting. The cage contains one 100 × 20-mm Petri dish lid in each of the back corners of the cage to hold the green leaf food and one Petri dish lid toward the front of the cage to hold tri-sulfa bran (see below).

Each cage (both large and small) sits on top of two parallel metal rails. The metal rails are elevated 4 inches above a sealed wooden bench. Cages have a wire-mesh bottom with no substrate in the cage. Newspaper or similar substrate is placed under the metal rails (below the cage) to collect fecal material and organic matter. A heat light rests on top for warmth, centered over the vertically hung piece of wire mesh. Heat lights have a 10-inch-diameter reflector hood with a ceramic base and a 75-W incandescent light bulb. The fluorescent room lights and the heat lights are on a cycle of 14 hours light:10 hours dark (on at 6 am, off at 8 pm). The grasshopper room also receives ambient light through windows.

All fecal material that is lying on the bottom of the cage itself is removed daily. The newspaper underneath each cage is replaced weekly with fresh newspaper. Long sheets of general purpose plastic line the top of the wooden bench beneath the newspaper. No chemical agents are used in the grasshopper room without prior approval from the principal investigator (PI), veterinary staff, and the Department of Animal Care and Technologies management. Offensive grasshopper odors can be minimized by simply removing fecal matter routinely. Cages are sanitized at intervals after the animals are removed. The cages may be run through a cage washer or simply be hand-washed with a hypochlorite or disinfectant solution and rinsed thoroughly with clean water.

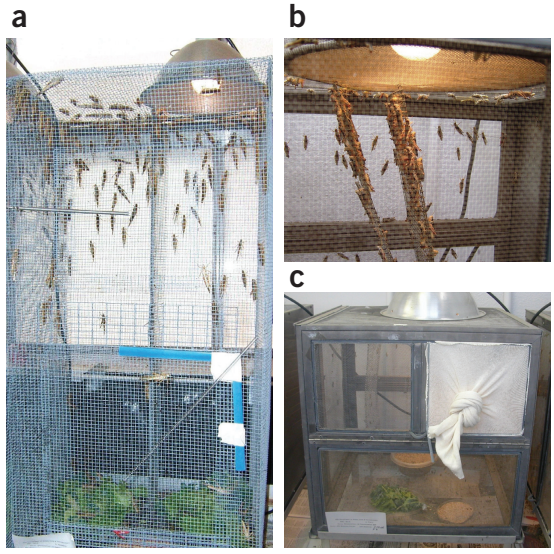

FIGURE 2 | Typical holding cages for grasshoppers. (**a**) Single-compartment caging unit for adult grasshoppers. (**b**) Grasshoppers on wire mesh hung within cage. (**c**) Cage for newly hatched grasshoppers with 1/16-inch diameter fine-mesh and a 7 × 7.5-inch opening closed off with an open-ended cotton sleeve.

Food

All grasshoppers are fed a 1:1 ratio of kale and green leaf lettuce daily. Kale leaves are removed from the stem and lettuce leaves are removed from the hub and torn into smaller pieces. Food is placed on 100 × 20-mm Petri dishes and is available *ad libitum* (**Fig. 3**). Uneaten food is removed from the cage at the beginning of the following day. The amount of fresh greens used daily is adjusted to ensure a small excess.

In addition, the grasshoppers always have dry food available in a similar 100 × 20-mm Petri dish (**Fig. 3**). A diet of greens alone, supplemented only by water, would likely be inadequate to provide for all the nutritional needs of the grasshoppers; thus, we also provide wheat bran. A nutritionally similar, and perhaps more convenient, food source for animal facilities is a pulverized block of commercial rodent chow (*e.g*, Teklad 8604, Harlan Teklad, Madison, WI). To reduce bacterial, fungal, and protozoan infections in the grasshoppers, the dry food source is treated with a combination of sulfa drugs (ref. 16): 0.9 kg of dry diet is spread out in a large bin and sprayed to surface wetness with a mixture of 0.6 g sulfathiazole, 0.4 g sulfapyridine, and 0.5 g sulphamethazine dissolved in 100 ml water. The bran is 'hand-tossed' (gloved hand) to keep it from clumping and localizing the tri-sulfa. After the diet has dried (overnight) it can be kept in a sealed container and refrigerated at 4 °C. Enough is preserved to supply the grasshoppers for a month. Larger quantities of bulk stock may be prepared and stored frozen at –20 °C. We have successfully used feed prepared and stocked in this manner for three months. To maintain hydration, kale and lettuce are sprayed with tap water prior to placement in the Petri dish. The tri-sulfa dry diet is lightly misted as well. Grasshoppers are not given a water dish.

Egg collection and care

Grasshoppers are provided with plastic cups containing commercially available 'play' (sandbox) sand in which to lay eggs (**Fig. 3**). The cups are placed with the adult grasshoppers beginning about one week after they become adults. Egg cups may be made by filling a 1-quart plastic container with sand. The sand is moistened with water and the container left uncovered. Once the sand at the bottom appears moistened, no more water is added. Care is taken to avoid adding too much water such that 'puddling' occurs at the surface. Should this happen, the cup is discarded.

FIGURE 3 | Adult grasshoppers in cage with 100 × 20-mm Petri dishes of greens (kale and lettuce) and dry food. Suitable forms of dry food include wheat bran and commercial rodent chow.

Two egg cups are put in a cage. Egg cups are removed from the cage after 3–7 pods are in the cup (usually this only takes 1–2 days). The disturbed sand surface will evidence deposition of pods. Any fecal debris is removed and the cups are covered with an appropriately sized Petri dish. The cups are then placed in styrofoam Hovabator incubators (GQF Manufacturing Co., Savannah, GA) with a constant temperature of 88–90 °F (31–32 °C), which has resulted in reliable hatch dates. The egg cups need to be watered slightly to keep the eggs from drying out, although too much water can lead to problematic fungal growth. It is possible to water the cups with a 1% aqueous solution of dyhydroxybenzoic acid to reduce fungal growth. The eggs hatch after 2–3 weeks. All stray eggs (those not laid in egg cups) are removed. These eggs are destroyed by freezing and then discarded. Used egg cups may be emptied of the sand matrix and washed in a 10% bleach solution. The sand is never reused.

S. americana hatches in about 21 days. *M. femurrubrum* hatches in 14–20 days. In rooms not maintained at constant warm temperatures, hatch time can range from 19–28 days for *S. americana* and 14–24 days for *M. femurrubrum*. Many species of grasshopper 'over winter' as eggs in an arrested state termed 'diapause.' This state frequently requires a period of exposure to cold temperatures or the eggs will not hatch. *M. femurrubrum* require a diapause at ~55 °F (13 °C), but not below 40 °F (4 °C), lasting more than four but less than twelve months. Although *S. americana* may be cooled for a diapause, our experience is that there will be less than an optimal hatch because *S. americana* over winters in an adult, not egg, diapause. The slowing (or cessation) of development of cooled *S. americana* eggs is a cold-induced quiescence.

Newly hatched grasshoppers are quite small (10 mg) and cannot be kept in the wire mesh cages with large openings. Thus, the egg cups are put into cages with 1/16-inch diameter fine-mesh with 18-inch–cubed aluminum on five sides. A solid plate of aluminum is placed on the top side to prevent debris from falling into the cage. The front of the cage has a 7 × 7.5-inch opening closed off with an open-ended cotton sleeve (**Fig. 2c**). The sleeve is tied in a knot to prevent the grasshoppers from escaping. The inside of the cage contains either one or two vertically hung ¼-inch diameter pieces of wire mesh suspended under the heat lamps for the grasshoppers to climb on. The pieces of mesh are attached to the bottom and top of the cage using metal ties. The cage has two Petri dish lids, one for holding greens and one for holding tri-sulfa bran. They are fed as the adults, but eat less.

The young need to be transferred to the larger cages at the fifth or sixth instar because the fecal pellets accumulate in the smaller cages. Instars 1–3 are especially sensitive to dehydration and temperature extremes. It is not abnormal for 50% of the hatchlings to die before reaching the reproductive stage. If the die-off is much higher than that, the cause should be investigated. The size range for intermediate stages of development (first juvenile to adult) are shown in **Figure 1b**.

Health checks

All cages are checked daily for injured, ill, or dead animals. Attention should also be paid to instar animals for signs of infection (such as nematodes protruding from the anus). Cages containing injured, ill, or more than 10 dead grasshoppers (or 5–10 % of the population) are labeled with a red cage card and reported to the clinical veterinarian. Since grasshoppers will cannibalize dead or dying cage mates, dead bodies should be removed daily to reduce the possibility for any cross-contamination in the event of an infection. If neglected, this can lead to major disease problems. For all non-health–related issues, orange cage alert cards are used to communicate with the PI. All events are recorded daily on forms that document feed changes, cage care, room temperatures, photoperiod, and other observations.

Occupational health and safety

There are some health hazards for those care staff assigned to the grasshopper colony. It is not uncommon to develop respiratory allergies or contact dermatitis, perhaps in response to something derived from the cuticle (exoskeleton) (see http:www3.imperial.ac.uk/occhealth/guidanceandadvice/workingwithinsects). A dust-particle (N-95) mask, gloves, and sleeve covers are required for anyone working extensively with the grasshoppers. In addition, it is important for staff to wash their hands after working in the room.

CONCLUSION

Colonies of nontraditional species do not normally comprise a significant proportion of a central ARF's

census, yet most ARFs will at some time or to some extent engage in the husbandry of unusual species, some not covered by regulations directed toward vertebrate species. Nevertheless, insects present an interesting challenge. Though perhaps historically kept by faculty investigators within their assigned spaces, turnovers of staff and use as instructional species without faculty assignment, as well as retention as institutional resources, provide compelling reasons for central maintenance of colonies. Furthermore, the necessary and sometimes daily attention to schedules and environmental parameters place the responsibility appropriately within the scope of the ARF. Costs can easily be translated from 'tech time' and supplies to per diems for services. Given the number of animals that may be generated within a relatively short time and held in a single caging unit, costs per animal are quite small. In the end, these species receive quality, humane care under the informed eye of dedicated care technicians and veterinary staff. In return, the ARF gains a somewhat unique satisfaction from the successful maintenance of unusual species.

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COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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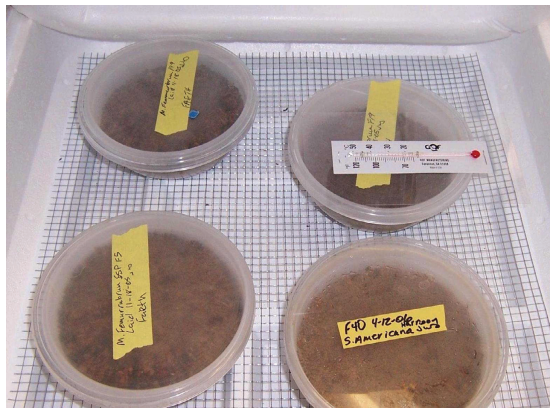

FIGURE 4 | Captive grasshoppers lay eggs in plastic cups, which are then stored in an incubator until hatching.

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