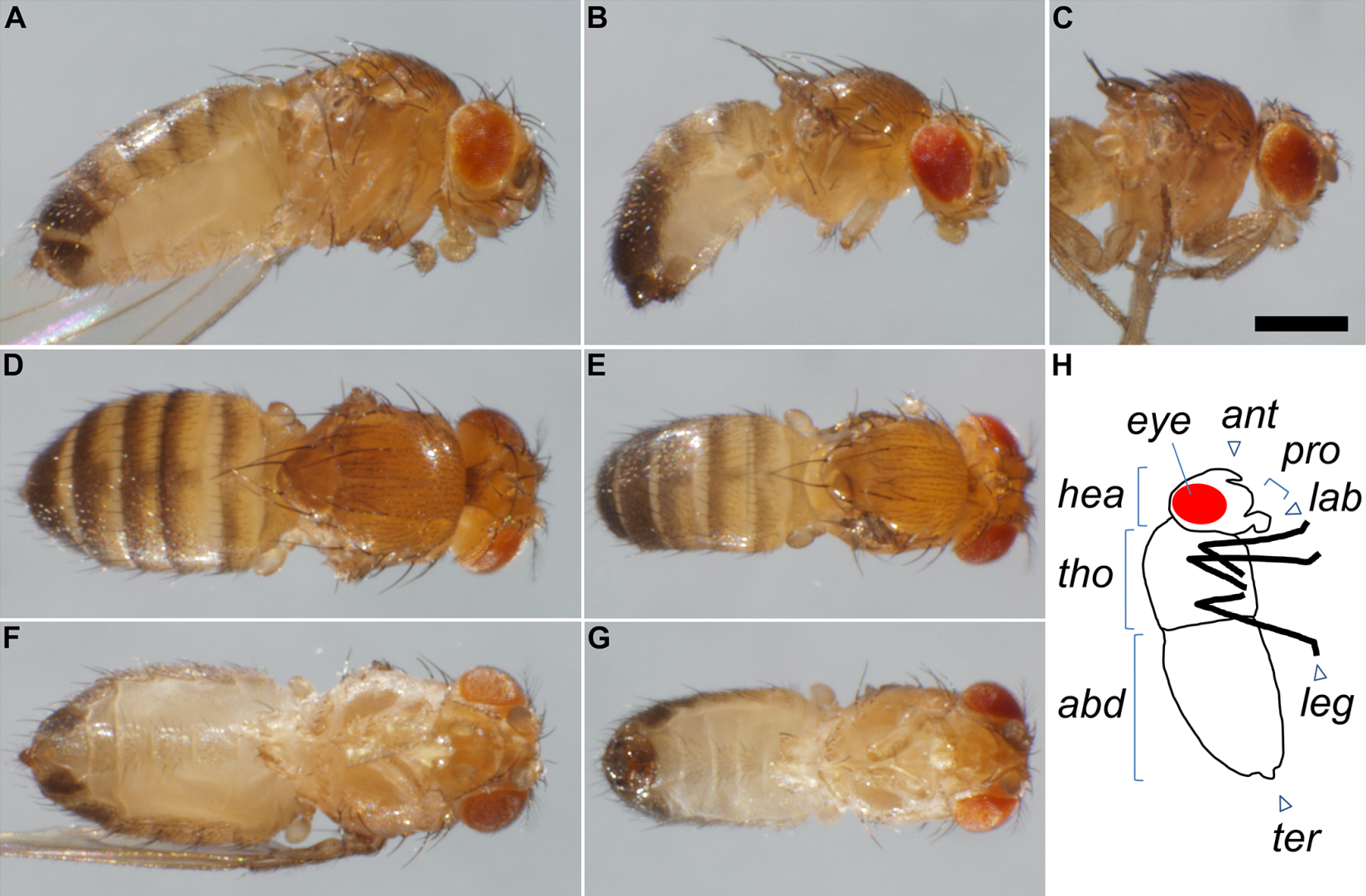
**Fly Behavior Observation Screen Handout**

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This exercise demonstrates many behavioral phenotypes by activating different sets of neurons in the brain, using 16 fly strains. The following figure shows the features of flies relevant to crossing flies for this exercise, and to the observation of behaviors that use different body parts:

Features of flies. Females (left column) and males (center column) viewed from side (*A, B*), top (*D, E*) and bottom (*F, G*), showing that males are smaller, have more black on the end of the abdomen, and have darker genital structures than females. (*C*) Some fly strains may contain the TM3 balancer, with a mutation causing a “stubble” phenotype. Note the shorter bristles on the back than in (*B*). After crossing a *GAL4* strain carrying TM3 to *UAS*-*TrpA1*, the progeny will be sorted under anesthesia to discard any flies carrying the stubble marker. Scale bar: 0.5mm. (*H*) Diagram of fly body parts, viewed from side. Abbreviations, in alphabetical order: *abd*: abdomen, *ant*: antennae, *eye*: eye, *hea*: head, *lab*: labellum (lips), *leg*: legs, *pro*: proboscis, *ter*: terminalia (which include the anus and genitals, and in females, also the ovipositor, an egg-laying tube that can be extended), *tho*: thorax.

*For the behavior exercises, training videos are available on YouTube, or may be provided by your instructor.*

Training videos (http://www.youtube.com/playlist?list=PL2D0slyugueK6E9UKPqfe-fdQbiVHnlCz):

Start with the introductory video (**Chambers.mp4**, with audio) showing how to load flies into the chambers and observe them.

Next, watch the demonstration of control flies (**Normal.mp4**, no audio).

While cooling off outside the warm room in between experiments, watch all the rest of the training videos to learn the different behavioral phenotypes. (There is no audio for the behavior videos.)

There are example videos for most but not all of the phenotypes on the list. Some phenotypes are hard to see in the videos but are much easier to see by eye.

Observing behavior:

Familiarize yourself with the descriptions of phenotypes in this handout. Also in this handout are behavior checklists, one for each of the 16 crosses.

Load flies into a pre-warmed chamber, bring into warm room, start timer, observe for 5 min. On checklist, note line name and check off phenotypes seen. Fill in any additional notes, if you can. First watch control flies to get a sense for the normal spectrum of behavior. When screening, only check off phenotypes that aren't normal, or that occur at an abnormal frequency. Note any gender differences. If possible, provide penetrance information as well: instead of just filling in check marks, fill in an estimate of how many flies were doing each phenotype. You can come up with your own way to score penetrance and discuss advantages and disadvantages.

Check yourself against the phenotypes other students see in these lines. Discuss possible reasons for any discrepancies.

Screen all 16 GAL4 lines.

|  |  |
| --- | --- |
| PHENOTYPE: | DESCRIPTION: |
| Abdomen - Abnormal | Anything abnormal about the abdomen that doesn't fit into other categories. |
| Abdomen - Curled | Abdomen is curled. |
| Abdomen - Elongated | Abdomen is narrower and elongated. |
| Abdomen - Held to One Side | This is subtly different from wagging abdomen from side to side; here the abdomen is only moved infrequently. |
| Abdomen - Moving Up and Down | This can either be slow movements, or fairly high frequency vibrations up and down. |
| Abdomen - Pointed | With or without ovipositor extension, which should be scored separately. Abdomen is not abnormally narrow; if it is, record as Elongated. |
| Abdomen - Rounded or Contracted | The abdomen can contract repeatedly in a rippling fashion, it can be lumpy looking, or it can be contracted at the base but not at the tip. |
| Abdomen - Wagging Side-to-Side | This is subtly different from holding abdomen to one side; wagging would be scored if the abdomen moves from side to side regularly. |
| Grooming - Abdomen | Grooming of the abdomen by the hind legs. |
| Grooming - Antennae | Hard to see because it happens rapidly. Higher magnification helps. Leg sweeps are concentrated on the front of the head. |
| Grooming - By Hind Legs | Some flies do excess grooming of one body part (e.g. abdomen), but there are also several lines that do excess grooming of all the body parts that they clean with their hind legs. Could count grooming of wings, abdomen and terminalia separately, but some lines show a general excess of grooming by the hind legs. |
| Grooming - Head | Grooming of the head by the front legs. |
| Grooming - Leg Rubbing | Refers to rubbing legs against each other, not against other body parts. |
| Grooming - Proboscis | Grooming of the proboscis by the front legs. Can occur with proboscis extended or not extended very much. |
| Grooming - Terminalia | Grooming of the fly's rear (terminalia) by the hind legs. Can occur with or without the terminalia being extruded. |
| Grooming - Uncoordinated | Flies may be generally uncoordinated, flies may try to groom a particular body part but legs fail to reach, or legs may fail to meet when trying to rub them together. |
| Grooming - Whole Body | Grooming occurs at fairly high frequency in controls, so excess grooming can only be determined with experience. Indicators can be that it would occur in many more flies than usual, or increases in frequency over time in the warmth, etc. But note: can also be due to flies housed in wetter vials. |
| Grooming - Wings | Grooming of the wings by the hind legs. |
| Head - Antennae Held Out | Antennae are extended. Subtle, have to compare to normal antennal posture. |
| Head - Bent Down | Head is held down. May notice that you can see the neck when looking from above, whereas normally you can't. |
| Head - Nodding Yes | Head nods up and down. |
| Head - Shaking No | Head swivels side to side. |
| Locomotion - abnormal | Any abnormal locomotion that doesn't fit into other categories. |
| Locomotion - Flight and Jumping | Excess flight or jumping. |
| Locomotion - Hyperactive | Can involve running, flying, and jumping. Has to be assessed in context of regular activity levels of controls. |
| Locomotion - Locomotion Reduced | Sometimes standing totally still, but could also just be reduced speed of walking compared to controls. |
| Locomotion - Walking Backwards | Not necessarily walking backwards in a straight line, some just take a few steps backwards. |
| Locomotion - Walking in Circles | Walking in circles. |
| Locomotion - Walking Sideways | Not necessarily walking sideways in a straight line, some just take a few steps sideways. |
| Posture - Abnormal | Any abnormal posture that doesn't fit into other categories. |
| Posture - Falling Over | Flies falling over. |
| Posture - Paralysis | Immobile, lying on side or back. If there's any movement left, categorize as "falling over" instead. |
| Posture - Seizing | Flies showing seizures, lying on side or back. |
| Posture - Shaky or Uncoordinated | Flies look shaky or uncoordinated. |
| Posture - Stance or Gait Narrow | All six legs are held closer to the body than usual. Indicators include tighter angles of the joints and legs generally held closer to body. |
| Posture - Stance or Gait Splayed | All six legs are held farther from the body than usual. Indicators include more open angles of the joints, body held closer to surface, and walking with leg segments flattened instead of walking on tips of legs. |
| Proboscis - Constitutive Extension | The proboscis is extended constitutively (consistently). |
| Proboscis - Courtship Licking | The male uses his proboscis to touch the rear of the female. |
| Proboscis - Extension When Hind-Leg Grooming | The proboscis is extended during grooming by the hind legs. (These two behaviors frequently occur together). |
| Proboscis - Holding Drop on Labellum (lips) | Sometimes occurs on its own; sometimes found with spitting on surface. |
| Proboscis - Labellar Spreading | Labellae (lips) are either held open constitutively, or are opened and closed. Easier to see at higher magnifications. |
| Proboscis - Repeated Extension | The proboscis is extended repeatedly. |
| Proboscis - Spitting on Surface | Liquid is extruded from the proboscis and deposited on the ground. |
| Proboscis - Touching to Surface | Can be with proboscis extended, or not extended (flies can bend over to touch proboscis to surface). You can tell the difference between this and just proboscis extension if the fly is bending forwards. |
| Social - Abnormal | Any abnormal social phenotype that doesn't fit into other categories. |
| Social - Female-Female Aggression | Aggression can include lunging, boxing, tussling, wing threat, or fencing. |
| Social - Female-Female Courtship | Female courtship is usually less pronounced than male courtship, so may not include as many of the components of orienting, following, tapping, licking, wing song, or attempted copulation. Control females do not usually follow other flies, so we note that here as a possible courtship-related phenotype. |
| Social - Female-Male Following | Females following males. |
| Social - Inter-Gender Aggression | Aggression can include lunging, boxing, tussling, wing threat, or fencing. |
| Social - Male-Female Courtship | Courtship can include any or all of the following: orienting, following, tapping, licking, wing song, attempted copulation, or copulation. We include courtship licking as a separate category, but that is a subcomponent of courtship. Courtship may or may not include licking. |
| Social - Male-Male Aggression | Aggression can include lunging, boxing, tussling, wing threat, or fencing. |
| Social - Male-Male Courtship | Courtship can include any or all of the following: orienting, following, tapping, licking, wing song, attempted copulation. |
| Social - Reduced Social Inhibition | Control flies usually try to avoid bumping into each other by turning away when they encounter another fly or by putting out a leg(s) to fend off another fly. "Reduced social inhibition" occurs when flies show less reluctance about bumping into each other or when they walk on top of each other. |
| Terminalia - Eggs Extruded | Can be eggs laid (deposited on surface), or just eggs protruding partway from ovipositor. |
| Terminalia - Liquid Defecation | Can happen quickly; might be easier to monitor by numbers of spots of excrement on surface. |
| Terminalia - Male Extended | Male genitals (terminalia) are extruded. |
| Terminalia - Ovipositor Extended | Female ovipositor is extended. |
| Terminalia - Solid Defecation | Can happen quickly; might be easier to monitor by numbers of spots of excrement on surface. |
| Wings - Deformed | Wings misshapen (developmental defect) |
| Wings - Flapping | More frequent movements than wing flicking. |
| Wings - Flicking | Faster movements of wings than flapping. |
| Wings - Held Down | Wings out to sides but held down towards surface. |
| Wings - Held Out to Sides | Wings held out to sides laterally. |
| Wings - Held Up | Wings held up. |
| Wings - Single Extended | One wing held out to side. |

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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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| **LINE:** | |  |  |  |  | **LINE:** | |  |  |  |  |
| **Abdomen** | | **Locomotion** | | **Social** | | **Abdomen** | | **Locomotion** | | **Social** | |
|  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |  | Abnormal |
|  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |  | Curled |  | Flight and Jumping |  | Fem.-Fem. Aggression |
|  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |  | Elongated |  | Hyperactive |  | Fem.-Fem. Courtship |
|  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |  | Held to One Side |  | Locomotion Reduced |  | Fem.-Male Following |
|  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |  | Moving Up and Down |  | Walking Backwards |  | Intergender Aggress. |
|  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |  | Pointed |  | Walking in Circles |  | Male-Fem. Courtship |
|  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |  | Rounded/Contracted |  | Walking Sideways |  | Male-Male Aggression |
|  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |  | Wagging Side-to-Side | **Posture** | |  | Male-Male Courtship |
| **Grooming** | |  | Abnormal |  | Reduced Social Inhib. | **Grooming** | |  | Abnormal |  | Reduced Social Inhib. |
|  | Abdomen |  | Falling Over | **Terminalia** | |  | Abdomen |  | Falling Over | **Terminalia** | |
|  | Antennae |  | Paralysis |  | Eggs Extruded |  | Antennae |  | Paralysis |  | Eggs Extruded |
|  | By Hind Legs |  | Seizing |  | Liquid Defecation |  | By Hind Legs |  | Seizing |  | Liquid Defecation |
|  | Head |  | Shaky/Uncoordinated |  | Male Extended |  | Head |  | Shaky/Uncoordinated |  | Male Extended |
|  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |  | Leg Rubbing |  | Stance or Gait Narrow |  | Ovipositor Extended |
|  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |  | Proboscis |  | Stance or Gait Splayed |  | Solid Defecation |
|  | Terminalia | **Proboscis** | | **Wings** | |  | Terminalia | **Proboscis** | | **Wings** | |
|  | Uncoordinated |  | Constitutive Extension |  | Deformed |  | Uncoordinated |  | Constitutive Extension |  | Deformed |
|  | Whole Body |  | Courtship Licking |  | Flapping |  | Whole Body |  | Courtship Licking |  | Flapping |
|  | Wings |  | Extension w/Hind Groom |  | Flicking |  | Wings |  | Extension w/Hind Groom |  | Flicking |
| **Head** | |  | Holding Drop on Lips |  | Held Down | **Head** | |  | Holding Drop on Lips |  | Held Down |
|  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |  | Antennae Held Out |  | Labellar Spreading |  | Held Out to Sides |
|  | Bent Down |  | Repeated Extension |  | Held Up |  | Bent Down |  | Repeated Extension |  | Held Up |
|  | Nodding Yes |  | Spitting on Surface |  | Single Extended |  | Nodding Yes |  | Spitting on Surface |  | Single Extended |
|  | Shaking No |  | Touching to Surface |  |  |  | Shaking No |  | Touching to Surface |  |  |
| **NOTES:** | |  |  |  |  | **NOTES:** | |  |  |  |  |
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