Chapter 4 : Stock Authentication

4.1 Stock Authentication by Morphological Characteristics

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Introduction

It is common for laboratories to rear several different species and/or stocks of the same species in one insectary. Often it is difficult to determine whether a colony has been contaminated, especially when the stocks appear identical. A PCR method to distinguish four anopheline species based on their 28S ribosomal subunit was developed as a quality control method to ensure contamination had not occurred between colonies (Kent et al. 2004). Although this method is highly specific, it can be very costly performing several PCR assays to detect a rare contaminant, so it is desirable to develop simple direct methods to verify colony purity.

Morphological discrimination of adults

It is not necessary to have extensive knowledge of mosquito identification to develop methods to keep stocks in order. A simple method to confirm identity is to develop 'local authentication standards' based on morphological characteristics of adults. These standards are not meant to distinguish your mosquito from all of those in the world but rather to distinguish the ones *you* maintain from one another. Therefore the standards are 'local'. The features can be described in very general language e.g. large adults, white knees, grey. Although these methods are not useful for members of cryptic species complexes like *An. gambiae*, it does work well when several different species of different appearance are maintained.

The local authentication standard consists simply of a chart that lists useful morphological characters that individually, or in some combination, distinguish all the species you keep. Its creation is simple. Remove several male and female members of each species and stun them in the freezer for a few minutes or anesthetize them by some other method. Place them side-by-side under a dissecting scope and scan prominent morphological landmarks (see below) to see if any differ. After selecting some candidate feature(s), scan larger numbers to ensure all individuals have the characteristic and it can be seen even in older individuals in which e.g. the scales may have rubbed off. An example of such a local standard is shown in Table 1.

Common Morphological Characteristics

- **Protarsi**: In *An. gambiae* and *An. farauti* you will usually find three white bands on the distal end of the protarsus. This characteristic is not seen in *An. dirus*, *An. freeborni*, or *An. quadrimaculatus*. **Figures 4.1.1-3**.
- **Metatarsi**: Unique, species specific, white banding patterns are often seen in *An. dirus* (white banding on the femur-tibia joint) and *An. albimanus* (prominent broad white bands on metatarsi). **Figures 4.1.4-6**.
- **All tarsi**: In some species the legs will appear spotted or speckled under magnification. This can be seen in *An. stephensi*, *An. dirus*, and *An. farauti*.
- **Abdominal banding patterns**: The ventral side of the abdomen can look very similar between species e.g. *An. stephensi* and *An. gambiae*. However, among others, there are various sizes of bands seen (e.g. *An. freeborni* have narrow transverse banding while *An. quadrimaculatus*, *An. atroparvus*, and *An. minimus* have wide transverse abdominal banding). **Figures 4.1.7-9**.
- **Halteres**: We have found that coloration of these structures is a good separation technique for a few strains. *An. dirus* and *An. farauti* both have halteres that are black ventrally and white dorsally. **Figures 4.1.10-12**.

- **Anterior wing margin**: Although the specific banding pattern is highly distinct, often the presence or absence of dark scaling on the wing margin is enough to distinguish between 2 species. **Figures 4.1.13-15**.
- **Palps**: Most anophelines have some banding on their palps. The number or width of bands or the lack of bands can be very diagnostic. **Figures 4.1.16-18**.

Protarsi

Figure 4.1.1. *An. gambiae*.

Figure 4.1.2. *An. farauti*.

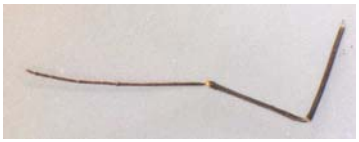

Figure 4.1.3. *An. quadrimaculatus*.

Metatarsi

Figure 4.1.4. *An. gambiae*.

Figure 4.1.5. *An. albimanus*.

Figure 4.1.6. *An. dirus*.

Abdominal pigment

Figure 4.1.7. *An. gambiae*.

Figure 4.1.8. *An. dirus*.

Figure 4.1.9. *An. albimanus*.

Halteres

Figure 4.1.10. *An. gambiae*.

Figure 4.1.11. *An. farauti* (dorsal side).

Figure 4.1.12. *An. farauti* (ventral side).

Wings

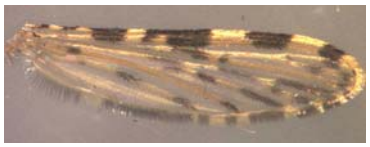

Figure 4.1.13. *An. gambiae.*

Figure 4.1.14. *An. albimanus*.

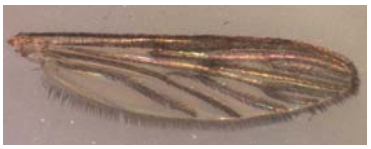

Figure 4.1.15. *An. quadrimaculatus.*

Palps

Figure 4.1.16. *An. gambiae*.

Figure 4.1.17. *An. quadrimaculatus*.

Figure 4.1.18. *An. dirus*.

Once you have made a checklist of which traits each species has, simply tabulate the results to see how they differ (**Table 4.1.1**). For example, if you have *An. stephensi* and *An. gambiae,* the two can be difficult to separate with most features but can be separated by the heavily spotted tarsi on *An. stephensi*. Likewise, *An. farauti* and *An. stephensi* can be separated from one another based on the presence of a ventrally black haltere as seen in *An. farauti*.

	STECLA	ORLANDO	F1	GA/AR/ME/QD	FAR1	STE-2
Character	albimanus	quadrimaculatus	freeborni	gambiae complex	farauti	stephensi
liberally spotted tarsi	N	N	N	N	Y	Y
haltere black ventrally	N	N	N	N	Y	N
prominent broad white band distal on metatarsi	Y	N	N	N	N	N
sooty dark wings	N	Y	N	N	N	N
prominent dark anterior margin wing pigment	Y	N	N	Y	Y	Y
mottled abdominal pattern similar to camouflage	Y	N	N	N	N	N

Table 4.1.1: A simple table of a few adult morphological characteristics useful to distinguish different species in laboratory settings.

Useful traits of immatures

Although not as useful as adult characters, there are some unique phenotypes that vary and are easily observed. The use of these phenotypes in conjunction with adult characteristics ensures strain purity.

Larval / pupal stripe: *Anopheles* larvae and pupae often have a unique white (**Figure 4.1.19**) or red (**Figure 4.1.20**) stripe on their dorsum (French and Kitzmiller 1964; Mason 1967), and its pattern and intensity varies. The *An. freeborni* F1 colony uniformly carries a white stripe phenotype which is especially noticeable in the pupal stage. The red stripe characteristic in *An. gambiae* is typically sex-limited to females. Not every *gambiae* stock will have this phenotype (*e.g. An. arabiensis* from Sudan do not express this whereas our *An. arabiensis* from Tanzania do) and the variation itself is a useful observation.

Figure 4.1.19. *An. freeborni* white stripe larvae (bottom) shown for comparison with *An. quadrimaculatus* (top).

Figure 4.1.20. *An. gambiae* larvae shown with (bottom) and without (top) red stripe characteristic.

Larval color: Mutants with differing larval color have been widely reported from *An. stephensi* in India as well as *An. quadrimaculatus* and *An. albimanus* (Seawright et al. 1985). Often these are genetic, but they also may be linked to diet. Culturing a pure-breeding variant-color colony can make separation of that colony from others quite easy (**Figure 4.1.21**).

Eye color mutations: Eye color mutants can be separated from wild-eye larvae based on their inability to melanize when reared in a dark or black pan (See Eye-Color Mutant Screening). Most larvae detect their environment and darken. Eye color mutants cannot discern their backgrounds so they will not melanize (**Figure 4.1.22**).

Collarless trait: Some larva will have a “collar” on the dorsum of the abdomen and some will not (**Figure 4.1.23**) (Mason 1967). Many wild strains are polymorphic for this trait. However, choosing those that either have the trait or do not to continue a colony can make it easy to quickly note a contamination event.

Larval postures when resting on the bottom: These are not definitive by any means. However, some species rest differently when compared side by side. *An. farauti* has a “U” shape, *An. gambiae* rest in an “L” shape, and some *An. atroparvus* larvae will appear to rest in a “?” manner (**Figure 4.1.24**). Other behaviors are distinct: *An. minimus* larvae cluster around the edge of the pan while very few will venture into the center. Once you learn what is customary for your strains, watch for changes.

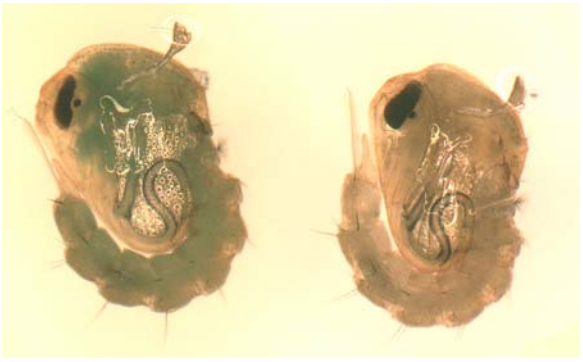

Figure 4.1.21. Examples from two *An. stephensi* strains carried simultaneously in an insectary, GREEN1 (left) and STE2 (right). GREEN1 was selected for a green mutation from the wild type strain. The green mutant is likely that of Suguna (1981).

Figure 4.1.22. *An. gambiae* (ASEMBO1) larva reared in a white pan (bottom) and a black pan (top) demonstrating the melanization capabilities of this wild-type strain.

Figure 4.1.23. *Collarless* trait shown on dorsum of *An. gambiae*. Compare the white pigment to collarless-minus individuals shown in **Figure 4.1.22**.

Figure 4.1.24. Curled larval resting posture seen commonly in a disturbed pan of *A. farauti*.

References

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