The Magic of Life Butterfly House

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Tropical Butterfly Pupae Care

With special reference to humidity

The successful emergence of butterflies from their pupae is key to a well-stocked butterfly display. There are a multitude of factors that affect pupal emergence, with disease and extreme temperatures being two of the most widely acknowledged problems. Pupal dehydration of tropical butterflies may well, however, be one the main reason for low emergence rates. This document covers all aspects of pupal care but it focuses on humidity, the importance of which remains underestimated.

Humidity and Pupae

Tropical rain forest countries typically have high humidities of 80-90% in the shade. A farmed tropical caterpillar pupates in the shade of a netted breeding cage at these high humidities. The thickness and permeability of its pupal skin is formed according to this prevailing humidity. When pupae travel from the tropics to the northern latitudes they are suddenly exposed to far lower humidities when their box is opened in the office. Typical values in the UK are often 30–50% relative humidity. The thin pupal skin adapted to the rain forest climate quickly starts losing water if left exposed in an office or outside of a pupal cage. Emerging butterflies form their straight wings by pumping fluid into very thin veins in the wings after emergence. The fluid in their abdomen is used for this but if the pupae has lost water due to dehydration, there is not enough of this fluid to completely fill the veins and the wings remain crinkled. A bit like blowing one of those party coiled blow-outs and not having enough air to fully extend it.

A paper written on dehydration in butterflies recorded 30% loss of water results in death due to dehydration. Far lower percentage losses of fluid e.g. 5-10% will result in malformations of wings. Even slight crinkling usually adversely affects flying and these crinkled-winged butterflies are rarely seen surviving more than two days in the flight area.

Background facts on humidity

Pupae have a very large surface area to volume ratio. This makes them especially prone to dessication as there is more surface area to loose water from per unit of volume. The smallest pupae such as Heliconiines, have three times the surface area/volume ratio of the larger pupae e.g. *Morpho*. It follows that the smallest pupae are worst-affected which is often what is observed in butterfly displays. Large Morphos rarely score dehydrated emergencies. *Heliconius* pupae are not only small but are especially sensitive to dehydration with thin pupal skins, *Philaethria dido* being among the most vulnerable of them.

Water vapour is a gas slightly lighter than air and will 'pool' at the top of the pupal cage and escape if it can. It can escape from any ventilation holes or gaps in the doors when a humidity gradient exists. Such ventilation holes can lead to low a humidity for some of the smaller species.

The evaporation of water in a normal room or air-conditioned office is greatly underestimated. e.g. a 2' x1' fish tank will loose a PINT of water in a day. The equivalent potential rate of loss for a pupa is high as it loses water from all its surfaces. As a test, roll up a piece of tissue paper into the shape of *Heliconius* pupa and soak in water and leave in the same places that the pupae are left in e.g. reception office and sealed pupal cage to gage an idea of rates of potential water loss. In our reception room, these assays can be dry within an hour. For this reason, we cover the pupae with two layers of damp kitchen paper as we glue them up on their rods.

Opening the pupal cage results in rapid loss of relative humidity. This should be minimised or the cage being damped down again after opening. In winter and long, cold nights, air heaters are on far more in the main display resulting in dangerously low humidities (also for the adult butterflies). Likewise in hotter weather, air ventilated in from outside can lower the flight display humidity. Humidity in a display mostly comes from the plant leaves and not the ponds which give off the same amount of water as the equivalent surface area of a pond.

Pupae bred in the actual flight area in northern lattitudes or by hobbyists in smaller greenhouses need no extra humidity control as their caterpillar and pupal skins have adapted to the lower humidity regimes in the first place. They therefore don't dehydrate as quick when left out of the pupal cage. Similarly, the pupae survive better at the lower temperatures they have grown acclimatised to. This is why butterfly houses and breeders

have reported differences in pupal success of home-grown Heliconiines compared with the same species imported from the tropics.

Diagnostics of Pupal Emergence Problems

Too Dry

- **Wings crinkled upon emergence**
- **Wings dry on emergence**
- **Abdomen size is thin or normal (butterfly body feels light)**
- **Small, yellow bubbles of fluid[1] sometimes present on wing veins**
- **% Successful emergence of small species is lower than the larger species**
- **% Successful emergence improves after 3 days[2]**

Too Cold/Too Hot/Too Wet

- **Wings crumpled or coarsely-folded on emergence, often fused together at tips**
- **Wings wet on emergence**
- **Abdomens normal size, not bloated, often wet**
- **Faded wing pigments (definitive for too cold or heat for pupae)**
- **No foul smell of bacteria**

Disease

- **Blackening of the pupal abdomen**
- **Dark rings between the pupal abdominal segments**
- **Emerged butterfly abdomen bloated with pale yellow fluid blisters.**
- **Emerged butterflies similar to ‘too cold’ with crumpled or coarsely-folded wings**
- **Emerged butterfly often has wet wings and/or abdomen**
- **Black foul-smelling fluid may be present**

Please see Appendix 1 for a pupal scoring sheet incorporating the above criteria. With the above table, it will enable a standardised comparison of pupae performance to be graphed out.

[1]Small, yellow bubbles of fluid (haemolymph) can sometimes been observed on the veins of the expanding wings of dehydrated butterflies. This occurs in cases of incipient dehydration when the wings have got stuck to the pupal skin and are ripped upon emergence. These can pump their wings up to the full extent but there is a question mark over how long these survive in the actual display afterwards. Usually the batch emergence will improve as the pupae are only slightly dehydrated.

[2]Percentage successful emergence improves after three to four days. This is because when a pupae is colouring up, enzymes digest the outer skin of the pupae so that it can easily break free from the pupa. A consequence of this is that pupae that are colouring up are many times more vulnerable to drying up (and physical damage) in transit than the other fresher pupae that still have thick, impermeable pupal skins. When glued on sticks in the puparium the coloured-up dehydrated ones are the first to emerge and have a higher chance of crumpled wings. After a few days successful emergence of butterflies increases due to the fact that they could withstand the journey and dry offices better. Whenever pupae emergence is worse at the beginning of the batch than at the end - low humidity and dehydration is implicated. This assumes the butterfly keeper, is controlling for humidity at their end.

Temperature Extremes – Too Hot / Too Cold

The combination of **faded wing pigments and wet wings** is definitive for pupae that have experienced temperature extremes I.e. too hot or too cold in transit. As to what temperatures tropical pupae can tolerate depends very much on the length of time the pupae is exposed to the extreme temperature. **Any temperatures below 18C and above 32C should be corrected for.** Excessive heat can do more harm quickly than bouts of excessive cold. Tropical imported pupae kept below 18C will have problems emerging. The African Papilionidae seem to have increased cold tolerance. Tropical equatorial pupae should NEVER be cooled or refrigerated to slow down their development. This will result in fatalities and wet, crinkled wings, especially with pupae close to emergence.

Disease

Any blackening of the abdomen; dark rings between the pupal abdominal segments indicates disease. In healthy pupae, only the wing pads darken up and not the pupa's abdomen. With freshly emerged but diseased butterflies, the wings or abdomen are often wet. The abdomens may also be bloated with pale yellow fluid blisters. This is especially so in Papilios that are virused. Caligos that are virused often emerge weak and the adult cannot grip on to its pupal skin sufficiently. Any signs of fluid especially black fluid or wetness which impede wing expansion are indicative of disease, or too cold or too wet conditions. Monarch pupae presenting **black blotches or dashes** on otherwise green pupa is indicative of O.E. *Ophryocystis elektroscirrha* – a protozoan disease specific to this family.

Dry dead pupae **with small round hole 1mm in diameter** in skin indicates parasitised by small parasitoid wasps. Tachnid flies parasitise other larger pupae such as Papilios and leave a larger rupture in the skin.

Practical Measures - How to control for humidity

- Maintain high humidities in the puparium i.e. 90% plus.
- Avoid hanging pupae in the flight area unless they are bred *In situ.*
- Keep pupal cages in complete shade as they are vulnerable to overheating in the sun. The outside can be covered in polystyrene and reflective foil to buffer heat extremes.
- Line the inside sides of the pupal cage with felt or coir matting. Irrigate these with drip hoses each day or manually water damp down with a couple of litres a day. In this way, it is not necessary to spray the pupae[1].
- Set thermostat of heater to 24-25C. Correct heat if it falls below 20C and rises above 30C for extended periods.
- Beware of heaters that dry the air in the pupal cage – this is commonly overlooked. Fan heaters should be avoided. One solution is to use a heating cable sandwiched between wet felt on the bottom and sides.

- Take measures if the pupae are wet for extended periods. If the pupal temperature drops significantly at night, excessive condensation will form on the pupae risking problems of wet emergence. Put more heating cables in to maintain set 24C temperature.
- Glue pupae up far enough apart to prevent them touching each other, as otherwise water or condensation collects on them.
- The butterfly keeper should assume there is some dehydration on arrival and take steps to avoid it. If it is a hot dry day, cover the pupae in lightly-sprayed kitchen paper while waiting for the glue to dry. Often this paper has completely dried in 15 minutes, demonstrating the potential danger of pupae being exposed in the office environment.

[1]Spraying pupae directly. You can spray pupae directly but not the ones that are colouring up (ie 1-2 day before emergence). Spraying coloured-up pupae will result in the water penetrating the wing pads resulting in the wings not expanding properly. Coloured up pupae are far more permeable than fresh pupae, making this stage the most vulnerable to both wetting and drying. For this reason, if the butterfly keeper randomly sprays their pupae in the pupal cage, it is likely that a percentage are colouring up and this will affect emergence.

For Heliconiines, if the main pupal cage is suspected of being too dry, a simple and inexpensive way of constructing a 100% humidity emergence cage is the following. Take a large cardboard or polysytrene box, cut a slit in the bottom and slide in a heating mat on a thermostatic plug at 25C, cover the mat in newspaper. Put some polystyrene cups on the bottom so any fallen butterflies don't get stuck to the wet paper. Cut notches in the box rim for the pupae sticks to hang on. Cover the top in two newspapers and cardboard on top of this so they extend over the rim and seal it. Then soak the whole set up with a hose till it is sodden through. Stick the thermostat probe in through the top where the pupae will be and then turn on the heat mat for at least one night – to check the temperature regime. Put the pupae in and re-soak the paper each day. This is how we do our Heliconiines even now as our main pupal cage can get too dry for a few of them. It has doors that overlap with a 1 cm gap which allows the water vapour to escape (we now block this gap). For Heliconiines, this cardboard box method has a 10% higher success rate than our already wet main pupal cage and it shows how important it is to be humidity aware.

Some people might be concerned with 95% humidity in pupal cages encouraging fungi and other diseases. Butterfly pupae generally emerge within a week of being put in the

pupal cage and are too transient to develop contagious diseases. Ones that develop disease are usually already infected from the caterpillar stage. Moth cocoons can take months to emerge and these would suffer under prolonged high humidity.

Using proper humidity control or the wet box method described above, we have achieved consistent rates of between 85-90% A1 emergence for Heliconiines imported directly from the tropics. Lower results than these especially when dehydration scores highly and the problem is located further down the supply chain.

Transport of Pupae - The egg-timer of dehydration

The haemolymph fluid may be lost at any stage in the supply chain of the pupae. Fluid loss in the pupa should be viewed as an egg-timer along the whole supply chain - as soon as a pupa leaves its sprayed delivery box it starts to dry out and the egg-timer is on. Whether this happens at the beginning, middle or end of the chain, the pupa is irretrievably damaged. We have tried soaking dehydrated pupae in water but it did not work. All pupae are vulnerable to dessication and warrant protection e.g. *Idea lecuonoe* and large Asian swallowtails can emerge dehydrated if not properly cared for.

Pupae are best transported sandwiched between two layers of cotton wool with the top side of the top layer sprayed 4-5 times ONLY before they are boxed up. This water will evaporate with time to fill the box with water vapour. **Note that it is important not to soak the cotton wool that the pupae are resting on, to avoid the problems associated with pupae being too wet.** We have had some imports that have over-reacted to our humidity advice and the pupae come in soaking wet! For heat extremes, polystyrene boxes covered in reflective foil dampen heat extremes. Exporters suffering from heat extremes on airport runways etc might consider using a double polystyrene box to buffer even further.

Some spiky pupae such as *Hypolimnas* and *Protogoniomorpha* (*Salamis*) from Africa and Asia very occasionally come in damaged especially the African species. It is important that these are individually wrapped right from the local farming stage all the way through to

delivery into the flight house. If they are jangling together in a transit box, they spike each other to death.

Importing pupae

In the importer's office in the temperate countries (or an exporter's air-conditioned office), pupae can start dehydrating as soon as they are out of their delivery box as they are often left for two days or more in very low 30-50% humidities. Pupae should be controlled for humidity in these offices with humidifiers. Temperature of 20-30C and 80-90% humidity is the correct environment; if there are no drips or condensation on the windows, it is too dry. Staff will constantly underestimate required humidity assuming 70% 'feels' high and hot enough.

A less effective sorting practice would be to drape damp flannels or towels over the boxes of incoming pupae so their exposure to dry office air is minimal. This method is prone to people letting the protocol slip. At the very least, the inside lid of each pupae box should be sprayed and sealed closed throughout the day and especially when the sorting process is finished for the day. Usually when importers send the pupae onwards to the butterfly houses, they do spray the inside of the box. Tropical equatorial pupae should NEVER be cooled or refrigerated to slow down their development. This will result in fatalities and wet, crinkled wings, especially as the pupae nears emergence.

The cVED rules requiring physical inspection mean that the box will be opened after customs clearance and much of the water vapour lost to the dry atmosphere in the airport. Vets should be asked to respray the cotton wool or inside of the lid of the box.

In conclusion, there are many ways to improve the health of the pupal stage. Compared with reducing the percentage of diseased pupae, raising humidity is the simplest and easiest of protocols to introduce. The goal should be to completely eradicate problems of pupal dehydration from the supply chain. Raising humidity awareness should be integrated as an important part of staff training for butterfly keepers.

For a pupal scoring sheet using the criteria adopted here, please see Appendix 1