Care of Butterfly Pupae - with special reference to dehydration

The successful emergence of butterflies from their pupae is key to a well-stocked butterfly display. There are several factors that affect pupal emergence, with disease and temperature being two of the most commonly recognised. Pupal dehydration may well, however, be the main reason for lower than expected emergence rates especially among small species. The solution is relatively simple although understanding the many factors that play a part in low humidity is complex and not widely recognised.

This document lays out the key points of how to maintain high humidities. It also puts forward a way of scoring pupal emergences to help narrow down the causes of deformed butterflies.

The pupal cage or puparium is used for maintaining temperature, preventing parasitoid wasps from escaping into the display and for keeping high humidities.

Humidity and pupae - Background information

A farmed caterpillar pupates in the shade of a netted breeding cage or in a netted bag in tropical rain forest countries with typical humidities of 80-90%. It forms its pupal skin according to this humidity. In northern latitudes with far lower humidities sometimes descending to 50-60%, the pupal skin starts losing water as it cannot adapt to form a more impermeable skin. A 30% loss of water in Heliconius results in death due to mortality. Far lower percentage losses of fluid e.g. 5% will result in malformations of wings.

Pupae can't take on water, whereas adult butterflies can drink making pupae far more susceptible to dehydration.

Pupae have a very large surface area to volume ratio. This makes them especially prone to dessication. It follows that the smallest pupae are worst-affected. Heliconius pupae are not only small but are extremely sensitive to dehydration, Philaethria dido being among the most vulnerable.

Relative humidity is what counts. It is higher in colder temperatures as cold does not hold so much water vapour as hot air.

Water vapour is a gas and can escape rapidly from a puparium that is not air tight. Displays heated by air heaters have variable humidity too low for pupae farmed in the tropics. Pupae bred in situ are adapted to the drier humidity regime and require no extra protection.

Water vapour is slightly lighter than air and will 'pool' at the top of the puparium and escape if it can. Being a gas, it can escape from any ventilation holes or gaps in the doors when a humidity gradient exists.

Opening the pupal cage results in rapid loss of relative humidity. This should be minimised with small pupae. In winter and cold nights, air heaters are on more in the main display which substantially lowers relative humidity. Likewise in hotter weather, air ventilated in from outside can lower the humidity.

Getting hold of good humidity meters is difficult but an essential tool for the butterfly curator.
Humidity in a display mostly comes from the plant leaves which give off the same amount of water as the equivalent surface area of a pond.

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 a day. The equivalent potential rate of loss for a pupa will be higher, although clearly it has some water tightness, as it loses water from all its surfaces, ie it has a larger surface area/volume ratio. Roll up a piece of tissue paper into the shape of Heliconius pupa and soak in water and leave in your various places that the pupae are left 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 damp kitchen paper as they dry on their rods. These assays can be used to give a good idea of potential evaporation rates in your office and puparium.

Identifying causes of pupal emergence problems

Too Dry
The main symptoms of pupal dehydation are *dry, crinkled wings* upon emergence, *abdomen either normal sized or thin* (not bloated). *The wings are not wet*. The wings cannot fully expand as the butterfly has partially dehydrated and lacks sufficient fluid to pump in and completely fill its wing veins. Hence they remain crinkled. Even slight crinkling usually adversely affects flying and therefore feeding and survival.

Small, yellow bubbles of fluid (haemolymph) can sometimes been observed on the veins of expanding wings. 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.

Disease
Any *blackening of the abdomen; dark rings between the pupal abdominal segments* indicates disease. With freshly emerged but diseased butterflies, *the wings or abdomen are often wet*. The abdomens may also be *bloated with pale fluid blisters*. A healthy butterfly will emerge with dry wings and abdomen. Any signs of fluid or wetness which impede wing expansion are indicative of disease, or too cold or too wet conditions.

Disease is out of the butterfly curator's hands but is often a result of too many caterpillars in the sleeve or host plant and not enough ventilation.

Too Cold/Too Hot/Too Wet
These symptoms are similar to diseased pupae. *Wet fluid on wings* prevents them from expanding especially on the *wing tips which can appear constatinered flat together.* There is no foul smell of bacteria. Pupae that have been exposed to either too hot or too cold emerge with *faded wing colours* (blanched). The combination of faded pigments and wet wings indicates temperature extremes in transit.

Tropical imported pupae kept below 20C will have problems emerging. Monarchs are an exception as they overwinter on the frost line in Mexico and have an inherent cool tolerance. Some of the African Papilionidae may also have some tolerance. **TROPICAL PUPAE SHOULD NOT BE COOLED OR REFRIDGERATED** to slow down their development. This will result in fatalities and wet crinkled wings.

Conclusion: Any of the following factors observed point to pupal dehydration.

i) dry, crumpled wings with no signs of wetness on the body, abdomen thin to normal sized.
ii) smaller pupae emerging better than larger species
iii) pupal emergence improving after 3-4 days

Percentage emergence of well-formed butterflies often increases after three to four days in the display puparium. This is because when a pupae is colouring-up enzymes digest the outer skin of the pupae

facilitating butterfly emergence. A consequence of this is that colouring up pupae are many times more vulnerable to drying up on the journey than the other fresher pupae that still have thick impermeable pupal skins. When glued on sticks in the puparium the coloured-up ones are the first to emerge and have a higher chance of crumpled wings. After a few days the emergence of perfect butterflies increases due to the fact that they could withstand the journey better. Whenever pupae emerge worse at the beginning of the batch than later on - low humidity is implicated.

How to control humidity - a cursory spray is not sufficient

I recommend maintaining very high humidities in the puparium i.e. 90% plus. For Heliconids and smaller pupae I use completely sealed cabinet to prevent any further water loss that they will have incurred in the transport of the pupae. Such cabinets are best kept in complete shade as they are far more vulnerable to overheating from the sun. Alternatively the outside of the cabinet can be cover in thick polystyrene and coir matting which effectively reduces incoming heat. While very high humidity puparium might seem to run the risk of disease and fungi, I have not observed contagion of diseased pupae to healthy neighbouring pupae but I have had many pupae emerging scored as dehydrated. Most recommendations for keeping vent holes are for long-lived animals in a terrarium where fungi would inevitably be a problem. Pupae, however, generally emerge within a week of being put in the puparium and are too transient to develop fungus in this way. Ones that develop disease are invariably infected already in the caterpillar stage. Moth cocoons can take months to emerge and these should be given ventilation. We found that when we swapped Heliconid pupae to 100% sealed pupal cage that there was an improvement in emergence rates by about 10-15% and this is the technique that we now use. Previously a one cm gap in the pupal cage sliding doors and two ventilation holes was resulting in reduced humidity. Using these techniques, we have consistently achieved 90% emergence from Heliconius pupae which have been imported directly from abroad. In completely sealed puparium, it is important to avoid large temperature drops at night which otherwise cause condensation to form on the pupae giving problems. Also spraying the pupae in such setups is not necessary as the water will remain on the pupae too long and can affect emergence.

It is best to avoid leaving pupae outside of the puparium. The butterfly display can experience rapid changes in humidity depending on season and outside weather. In cold weather the heating is on far more and if a hot air heater or fan heater is used there will be a rapid reduction of relative humidity. In very hot weather the extraction fans bring in cooler dryer air that also lowers relative humidity within the display.

It is best if they remain sandwiched between two layers of cotton wool with the top side of the top layer sprayed 5-6 times before they are boxed up and sealed again till they are ready for glueing. This water will evaporate with time to fill the box with water vapour. The pupae should be placed in a humidity controlled and temperature-controlled puparium as soon as possible.

Spraying pupae directly. You can spray pupae directly but it is best avoided when they are colouring up. 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 which making this stage the most vulnerable to both wetting (and drying).

The best way to increase relative humidity in the puparium is to have the inside sides covered in water absorbent felt, coir matting (or even cardboard) and this be wetted twice a day with a couple of litres of water.

The butterfly curator should assume there is some dehydration on arrival and should take steps

to prevent further damage. I take no chances and cover the pupae in lightly sprayed kitchen paper while waiting for the glue to dry. Often the paper has completely dried in 15 minutes, indicating the potential for losing water that exists in an office environment.

Interaction factors of cold and heating the puparium

Heating the puparium can of course lower the humidity resulting in dehydration. Pupae emerge best at the temperature they pupated at e.g. 25C However in cooler displays at night, heating up the puparium to such high temperatures increases the chance of dehydration so setting the thermostat at 22C-24C is a safer option.

Likewise pupariums that are not heated and are kept very wet have the chance of water condensing on the pupae in cooler nights. This water would affect pupae close to emerging. For this reason it is essential that all puparium kept at high humidity be thermostatically controlled with heating wires/ mat beneath the felt.

Transport of Pupae - The egg-timer of dehydration
Scientists have found that Heliconius adults die at 30% dehydration and they found large differences between species. Dry crinkled wings results from not having enough haemolymph (body fluid) to sufficiently fill the wings veins to straighten them. Crinkling of wings via dehydration would happen at lower than 30% dehydration and possibly at as little as 10%.

Heliconius pupae and other small pupae are the most susceptible to dessication. Pupae left out overnight exposed to normal office air will result in dry crinkled adults. But really ALL PUPAE ARE VULNERABLE TO DESSICATION and warrant protection. I have witnessed *Idea lecuonoe* and large Asian swallowtails emerge dehydrated. Importantly the water may be lost at any stage in the supply chain of the pupae. Dehydration should be viewed as an egg-timer along the whole supply chain - as soon as a pupa is outside its sprayed polystyrene box the egg-timer is on. Whether this happens at the beginning middle or end of the chain is irrelevant.

In a central collecting depot pupae can start dehydrating as soon as they are out of the shade netting. Tropical cities have lower humidities as do air conditioned offices. Again pupae should be controlled for humidity in the depot as they may be there for some time. A good quality humidity meter is an essential tool to aid humidity control in such environments. In larger sorting operations, damp flannels or light towels can be draped over the boxes or the box lids constantly resprayed and sealed. Alternatively the pupae could be left in humidity-controlled rooms with a electronic humidifier with the humidity set at 90% i.e as high as the pupal cabinet although this might give problems with staff. The same counts for pupae wholesalers in temperate countries. Most exporters spray the foam/cotton wool of the pupae just before sealing the package. This is essential as the pupae will be in there for four to five days. IT IS IMPORTANT NOT TO SOAK THE COTTON WOOL. Too wet gives just as many problems as too dry. Five or six sprays of water would suffice for it to evaporate into the sealed box and maintain high air humidity. As soon as the box is open that air humidity is lost and it will need to be re-sprayed. Pupae should be

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

Pupae bred in the actual flight area of a butterfly house or by hobbyists need no extra humidity control as their caterpillar and pupal skins are adapted to the lower humidity regimes.

To conclude pupae in the whole supply chain are only safe when they are packed in a water tight box such as the standard polystyrene ones with 5 or 6 sprays of water on the inside of the lid before it is closed.