# 10 week summer placement with the department of Biosciences and the School of Information Systems, Computing and Mathematics

**By Esther Bamigboye**

**PROJECT NAME: IMAGING OF PCP IN *DROSOPHILA MELANOGASTER***

The duration of the project was from the 20th JUNE 2011- 28th AUGUST 2011 and took place at both the Heinz Wolfe and the St John's buildings.

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- **Mr Ville Rantanen**- University of Helsinki Computational Systems Biology CSB: image analysis

# Planar cell polarity (PCP) signalling in *Drosophila Melanogaster*: The beginner's guide

## Abstract

Planar cell polarity signalling is the mechanism through which a number of tissue types, such as epithelia, determine the polarity of their cells perpendicular to their cellular apical-basal axis. The PCP signalling pathway is constituted of the following modules; a global directional cue, a core module and tissue specific effector-modules. Within each module, a collection of proteins interact to translate instructions to the cell to determine the tissue's polarity. This occurs during the early stages of development. In *Drosophila Melanogaster* the 4 main tissues currently studied are the eye, bristles of the notum, the abdomen and the wing. When PCP signalling goes wrong, i.e. PCP gene mutations, it can lead to congenital disorders such as polycystic kidney, conotruncal heart defect and open neural tube defects in mammals (Jeffrey D. Axelrod and Claire J. Tomlin 2011). This is as a result of mutation in genes coding for the vital proteins of PCP signalling. PCP mutations have also been implicated in idiopathic pulmonary hypertension (Jeffrey D. Axelrod. 2009) and Usher Syndrome (Helen McNeil and Jeffrey D. Axelrod. 2002). This review focuses on the functions PCP signalling modules and the properties of the proteins which drive them to bring about the formation of a distal, orientated hair on the wings of drosophila.

## 3 for 1

*Drosophila Melanogaster* is a good model for PCP signalling because the PCP signalling in vertebrates has been conserved in drosophila**,** homologous genes (Jeffrey D. Axelrod 2009). PCP signalling was first discovered in Drosophila after the observation of a disruption of bristle order caused by spontaneous mutations of PCP genes (Helen McNeil 2010 citing Fahmy and Fahmy 1959). The trichome hairs, which usually emerge from the distal portion of the cell and point towards the distal part of the wing, were disorientated; with some hairs emerging from the centre of the cell or at variant distances from it depending on the mutation (Jeffrey D. Axelrod and Claire J. Tomlin 2011).

Mutations in different genes give rise to non-random patterns (Jeffrey D. Axelrod and Claire J. Tomlin 2011) such as the *dsh* gene as illustrated in figure 1b. This unique pattern is the result of a mutation in the *dsh* gene. The same can be said for mutations of other core module genes. A phenomenon termed domineering non-autonomy is observed outside clone mutants for some PCP genes, e.g. *vang* and *fz* (Adler PN et al 2000). Domineering non-autonomy refers to the effect mutant cells have on neighbouring cells; disrupting their cell polarity. This singularity has lead to

scientists believing a possible 'factor X' is responsible for the effect on non-mutant neighbouring cells by diffusing across from mutant cells (Jeffrey D. Axelrod and Claire J. Tomlin 2011). Research on Drosophila with mutations in different PCP protein coding genes is instrumental in figuring out the roles of many of its proteins.

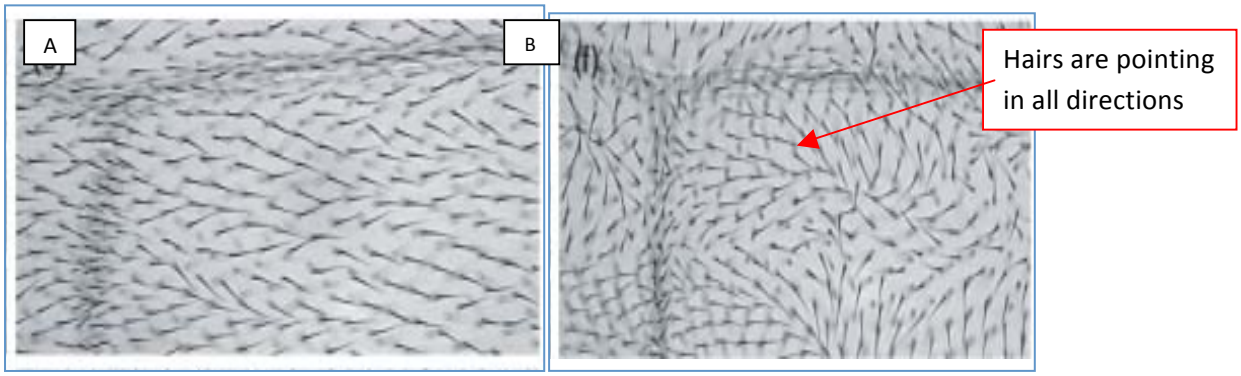


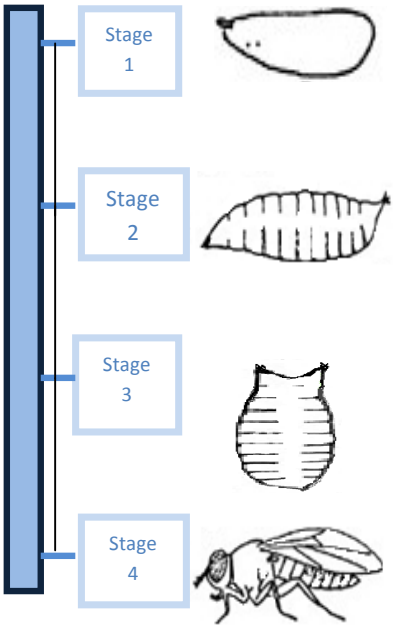

**Figure 1a** shows the wild type hair pattern in Drosophila wing. The hairs are all pointing towards the distal portion of the wing**. Figure 1b** shows the disruption of the wild type hair orientation due to a mutation in the Dishevelled (dsh) gene**.**

**It is generally accepted that PCP signalling is orchestrated by 3 modules**; a global directional cue, a core module and tissue specific effector-modules (Jeffrey D. Axelrod and Claire J. Tomlin 2011, Tree et al. 2002). Each module is driven by a set of proteins and RNA which interact to bring about a specified organisation of certain components within the cell. PCP proteins have been found to be involved is in other pathways: the canonical Wnt pathway, the hippo pathway (Sopko and Mcneil 2009, Peter A. Lawrence et al 2007) and the heterotrimeric G protein (HGP) pathway. These pathways are distinct from PCP signalling but have common components e.g. the *fz* protein. It is being debated whether PCP is actually composed of more than one pathway (Peter A. Lawrence et al).

## The drosophila life cycle *in vitro* and Pre-hair formation

Drosophila is a holometabolous insect, meaning it has four life stages - as an embryo/egg, a larva, a pupa and an adult. In laboratories D. Melanogaster, having the ability to develop at various rates depending on temperature, is cultured at 25°C or 18°C. They are housed in plastic or glass bottles, within which is contained a food medium constituted of; water, agar, dextrose, maize meal, dry yeast and Nipagin (an anti-fungal factor).

***Timeline: at 25C (*Source Micheal Ashburner 2000)**




***Embryo/egg***: A mated female is capable of depositing up to 100 eggs per day, which she lays on or in the food. The egg develops for 24 hours.

***Larva*****:** the egg develops into a 1st instar larva which immediately begins to feed on the medium. The next phase is a 2nd instar larva, the final being a 3rd instar larva. Both the 2nd and 3rd instar larvae eat through and into the medium where they remain for 4 to 5 days. At the end of this period, they emerge from the food and relocate onto the side of the bottle where they undergo pupariation.

***Pupa:*** A larval cuticle forms around the larva and develops into a pupal case. The larva goes from a milky tone to a darker tanned one. During this stage, which lasts about 4-5 days, metamorphosis occurs.

***Adult*****:** on day 12 an adult fly emerges, inflates it wings and loses excess fluid.

## The wing

There are 30,000 hair producing wing cells in each drosophila wing (Gregory M. Guild et al 2005). The hairs are the result of the aggregation of mostly actin fibres and a few microtubules (Helen McNeil and Jeffrey D. Axelrod 2002). The distal area of the wing produces hairs earlier than more proximal wing area. The pre-hair first emerges distally then becomes more central further into development (Helen McNeil and Jeffrey D. Axelrod 2002). The hairs are believed to aid in airflow across the wing (Gregory M. Guild et al 2005).

## Global cue

The global directional cue module proteins include:

| PROTEINS | Extra Information |
|---|---|
| **Dachsous (ds)** | Is a large atypical protocadherin like protein |
| **Golgi resident protein Four jointed (fj)** | Is was believed to be a novel trans-membrane protein may be cleaved and secreted, but recent research suggests it is a golgi kinase |
| **Atypical cadherins fat (ft)** | Is an atypical cadherin like protein tumour repressor. It has an extracellular domain (ECD) intracellular domain (ICD) |

**Table 1** shows a summary of known Global core module proteins and their properties. (Helen McNeil and Jeffrey D. Axelrod. 2002)

The global cue module couples the cellular polarity to cellular axis (David Strutt and Helen Strutt 2007). There are unresolved areas in the scientific knowledge of the Global cue module. It is known that the global cue proteins**, *ft, ds*** and ***fj*, AKA the *fat/ds/fj* cassette** (J.R. Barrow 2006), regulate PCP in all Drosophila tissue (Helen McNeil 2010). In the wing of Drosophila, global cue proteins spread across the tissue, unlike core proteins which are asymmetrically spread across the *cell.* Ds is highly expressed at the proximal end of the tissue and is absent from the distal part of the wing. *Fj* is highly expressed in the distal ends of the tissue and its concentration fades over the wing (to the proximal side). *Fat*, which is evenly dispersed through the wing, regulates the expression of *Fj* by binding directly to a transcriptive repressor, Atrophin. Yang et al 2002, suggest that *Ds* binding to fat inhibit *fat* activity. *Fat*, a large cadherin, functions as a receptor for *Ds*. *Fat* and *Ds* forms cis–dimers, *Ds* binding to *fat* encourages dimerisation and phosphorylation by the kinase, Dco (Sopko et al 2009). Kinase Dco binds to *dsh*, thereby linking the global cue module to the core module. There are opposing groups of researchers on the subject of whether the global cue module and the core module function in series or whether they are parallel (J. Casal et al 2006)

## The main core module

The main core module proteins are as follows:

| PROTEIN | Extra Information |
|---|---|
| **Strabismus/Vang-** | Putative trans-membrane protein with potential PDZ binding domain |
| **Frizzled (Fz)** | 7 trans-membrane protein, |
| **Flamingo/starry night-(Fmi/Stan)-** | Is a cadherin like novel 7-transmembrane cell adhesion molecule |
| **Diego (dgo)** | Thought to be cytoplasmic 6 Ankyrin repeat protein |
| **Prickle-spiney legs-(Pk)** | Novel LIM-domain-containing protein (it has 3 isoforms: Pk, M and Sple) which are produced by alternative splicing of RNA's |
| **Dishevelled (dsh)** | Is a cytoplasmic protein with PDZ,DEP and DIX domains |

**Table 2** shows a summary of know core module proteins and their properties (adapted from Helen McNeil and Jeffrey D. Axelrod 2002 and J.R. Barrow 2006)

The core module amplifies and stabilises sub-cellular asymmetry via a bistable feedback loop that is yet to be fully understood (David Strutt and Helen Strutt 2007, J.R. Barrow 2006). Core module proteins become localised to apical junctions early in development (Axel 2001, Das et al 2004). According to *Hannus et a, 2002*a B' regulatory subunit of PP2A, wider borst, is required to organise tissue polarity proteins to the proximal and distal ends of the cell. It is also needed to produce the polarised membrane outgrowth which later becomes the trichome hair

(*Hannus et al 2002*). The proteins are stored in vesicles from which they can be released to be distributed.

*Fmi*, a 7-transmembrane cell adhesion molecule, has an extracellular domain which contains multiple cadherin repeats. It can engage in homotypic cell-cell adhesion by association with other core proteins. *Fmi* adhesion is essential for PCP activity (Helen Mcneil 2010). During development, all the core proteins are evenly distributed throughout the cell. During pupal development, the core proteins, *fz, dsh, Dgo, vang* and *pk,* are asymmetrically distributed throughout the call. *Ds, fmi* and *fz* both are localised to the distal side of the cell before pre-hair formation; this is where the pre-hair is formed. At the proximal side *pk, fmi* and *vang* become concentrated (Jeffrey D. Axelrod and Claire J. Tomlin 2011). These proteins, located at the cell membrane, engage in cell-cell communication with adjacent neighbouring cell to establish polarity locally (Burak Y, Shraiman BI 2009).

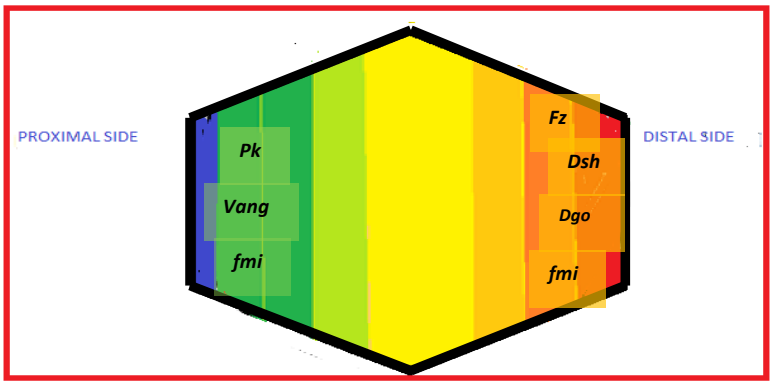



**Figure 2** shows the location of the core proteins, fz, dsh, Dgo, vang and pk, in the cell during pupal development. Fz, Dsh and Dgo are located at the distal side while Vang and pk are located at the proximal side. Stan is located at the proximal and distal ends of the cell**.**

## A summary of function

**Dishevelled**: is involved in the wingless and Wnt pathways; in which the frizzled protein is also involved (Richard Morgan et al. 2003, Roel Nusse at al 1994)

**Frizzled**: It is believed that *Fz* receives morphogenic information from upstream proteins such as the global cue protein cassette. Being a trans-membrane protein, it is involved in sell-sell communication with adjacent cells to regulate cellular polarity. Second, *Fz* is involved in a process of cell–cell communication with adjacent cells to locally co-ordinates cellular polarity. *Fz*, concentrated at the distal edge, is also required to invoke hair growth at the distal end of the cell. In

its absence, the trichome hair emerges at the centre of the cell. (David Strutt and Helen Strutt 2007).

**Flamingo**: associates with frizzled and dishevelled to direct the formation of a hair at the distal portion of the hair cell (Richard Morgan et al 2003)

**Prickle**: is localised at the proximal end; it binds to Strabismus which transports it to the membrane. It is also suggested that pk levels are regulated by strabismus (Rebecca Bastock et al 2003, Tree et al. 2002).

**Strabismus /Vang gogh**: is involved with the frizzled signalling/signal transduction pathway. Van Gogh mutants display domineering cell non-autonomy.

**Diego:** association with *dsh* and *pk* aids in amplifications of asymmetry the at proximal–distal cell boundaries.

As a result of the beehive arrangement and association between cells, the proximal proteins of 1 cell can attract the distal proteins of another cell to the distal membrane (Jeffrey D. Axelrod 2009). This is shown in figure 2 (from Burak Y, Shraiman BI 2009)

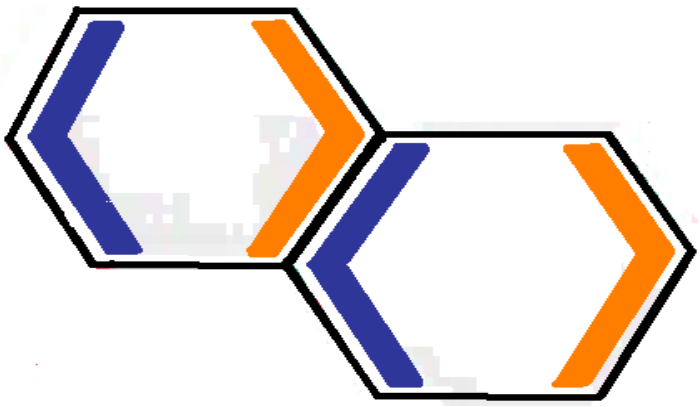


**Figure 3:** This image is a diagrammatic representation of the positions and interactions of the core proteins within the hexagonal cells of the drosophila wing cells. The blue bar represents the proteins; pk, vang and fm. The orange bar represents the core proteins dsh, fz, dgo and fmi

## Tissue specific effector-modules

Tissue specific effector-modules (in wing PCP)

| |
|---|
| • **Multiple wing hair (Mwh)** |
| • *fritz* |
| • Fuzzy (fy) |
| • Inturned |
| • Drok pathway (Rho-associated kinase) |

**Table 3** shows a summary of the tissue specific effector modules and their properties**.**

The tissue specific modules, in response to upstream modules, produce 'morphological asymmetry in individual tissues' (David Strutt and Helen Strutt 2007). Mutants of the inturned gene may have an extra wing, form multiple pre-hair at a variety of locations along the apical cell periphery or exhibit hair disorientation (Huiqing Zeng et al 2010, Jeffrey D. Axelrod and Claire J. Tomlin 2011). This module is not as well understood as the core module; current research is endeavouring to shed light on it (Huiqing Zeng et al 2010).

## How computational modelling fits into the picture

Computational modelling is an innovative instrument that is being used in the effort to figure out the inner working of the PCP pathway. The programme snoopy is an example of a tool being used to create Petri nets. These Petri nets, designed by the German mathematician and computer scientist Carl Adam Petri (1926- 2010), are used to predict and illustrate the various possibilities presented in PCP signalling using knowledge discovered by biologists.

The PCP pathway is a vital one in mammals, this is supported by the consequences of mutations in PCP; polycystic kidneys and deafness to name a couple. A few of the issues that remain to be resolved are the identity of 'factor X', the definite number of pathways involved in PCP and the mechanism of action for the tissue specific effector module.

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## My role as a Research Assistant

As a Research Assistant my aims were to produce images of *drosophila melanogaster* wings to be used for computational analysis. My other aims included gaining experience in research techniques.

## Culturing: this section illustrates how the flies are breed for experiments

Food culture preparation:

**Safety warning: Wear lab coat and gloves!**

### Apparatus

- **Cotton wool**- to bung up test tubes and bottles
- **Test tubes**- to hold flies
- **Plastic bottles**- to hold flies
- **Beakers (400ml, 600ml and 1000ml):** for mixing and food distribution
- **Hob Cooker**- to cook food
- **Fridge**- to store food
- **Test tube racks**- to hold test tubes
- **Baskets**- to hold bottles
- **Scale**: to weigh ingredients
- **Stirrer**: to stir food mixture
- **Gloves**: for safety
- **Lab coat**: for safety
- **Measuring cylinder**: to measure IMS

**MIXTURE 1-** Ingredients

- 650mls of water
- 9 grams of agar
- 2.5 grams of Nipagin (anti fungal agent)
- 25ml of IMS (Alcohol)

**MIXTURE 2-** Ingredients

- 200mls of water
- 85 grams of maize meal
- 75 grams of Dextrose
- 15 grams of yeast extract

### [1]Instructions:

**Prepare bottles and test tubes ready for food to be poured in**

**For the first mixture:** Add 9ml of agar to 650ml of water in a 1L beaker and warm on a low flame on the hob. Dissolve 2.5 grams of Nipagin in 25mls of IMS, and then add to the already warming mixture. Warm until agar has dissolved; the mixture will come to a boil and have a clearer appearance. Make sure to regularly check mixture 1 whilst preparing mixture 2.

**For the second mixture:** while the first mixture is still warming up, dissolve 75grams of dextrose completely in 200ml of water in a 600 ml beaker. Add 15 grams of yeast extract and then slowly add the maize meal; make sure to avoid lumps by adding minute amounts of maize meal at a time. The mixture will be thick.

Once mixture 1 is ready, add mixture 2 and stir well. Whilst this new mixture is hot pour the food culture into the prepared test tubes and bottles. In the test tubes the food should be approximately 1cm in height and in bottles the food should be 1inch in height.

[1] Recipe taken from: **Laboratory culture of drosophila:** Micheal Ashburner. (2000). **Laboratory culture of drosophila** In: William Sullivan, Michael Ashburner, R.Scott Hawley *Drosophila Protocols*. New York, USA: Cold spring Harbor Laboratory Press. P585-595

Cover the food with blue roll and leave to cool overnight. The next day bung up the test tubes and the bottles with cotton wool. It is now ready to use or can be stored in the fridge.

**Breeding: Culturing:**

**Apparatus:**

- **Binocular microscope**
- **Paint brush**
- **$CO_2$ tank**
- **Bottle containing vegetable oil**

The fly stocks need constant care. Every morning all flies in a bottle are disposed of in a bottle containing vegetable oil. This is so mated females are removed from the stock; that way the genotype of the flies within a population are kept constant. This is the first part of virgin collection. In the evening all the flies in a bottle are emptied on to the surface of the extension of a binocular microscope. The flies that have hatched do not mate for the first 8-10 hours of life, 8 hours after the morning clearing out is when the second part of virgin collection is completed.

There is a tube which is connected to a $CO_2$ tank. The $CO_2$ is directed to the extension here; as $CO_2$ is denser than air it forms a film of $CO_2$ gas in the extension which holds the flies. This acts as a general anaesthetic for the flies. The males are gently separated from the females. These females are virgins. We know this because all of the adult fly population, which may have contained mated females, were removed in the morning. The females virgins are then stored in a labelled test tube with food. They are monitored for a few days to ensure they do not lay any eggs. If they do not, these female flies can be mated with males in test crosses. It is easier to predict the genotype of offspring when the genotypes of parents are known.

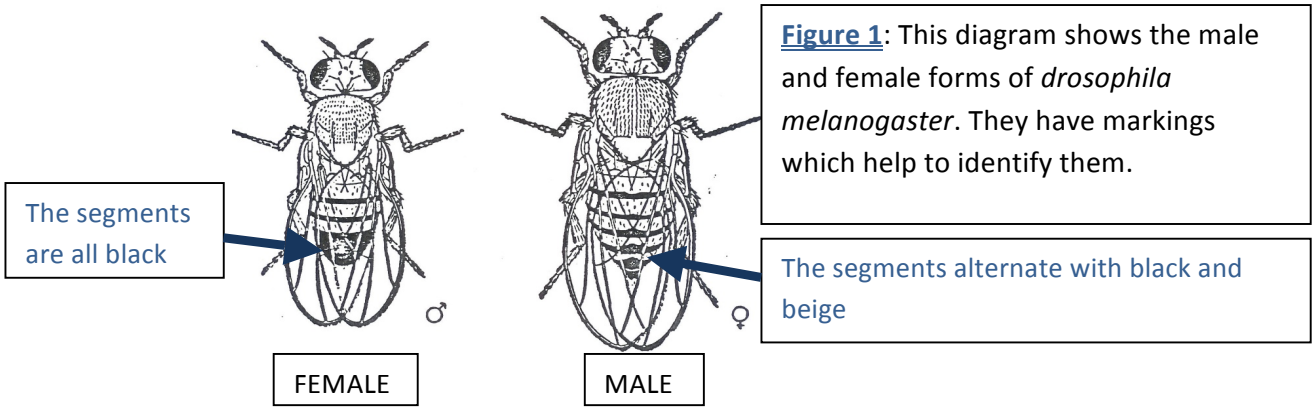


**Figure 1**: This diagram shows the male and female forms of *drosophila melanogaster*. They have markings which help to identify them.

Image taken from:
http://www.google.co.uk/imgres?q=drosophila+male+and+female+fly+wings&um=1&hl=en&sa=N&qscrl=1&nord=1&rlz=1T4DKUK_en-GBGB271GB272&tbm=isch&tbnid=U5_LynT3eiGXgM:&imgrefurl=http://chsweb.lr.k12.nj.us/psidelsky/FruitFlyStudy%2520Guide.htm&docid=vlAMh5IqojaDyM&w=1402&h=1051&ei=5I9zTuGFIoSg8QP0hLnZDQ&zoom=1&iact=hc&vpx=119&vpy=145&dur=52&hovh=194&hovw=259&tx=146&ty=132&page=1&tbnh=100&tbnw=133&start=0&ndsp=12&ved=1t:429,r:0,s:0&biw=1054&bih=458

## Dissections: Adult wing dissection

Apparatus:

- **binocular microscope**
- **paint brush**
- **$CO_2$ tank**
- **Slides and slip covers**
- **Bottle containing vegetable oil**
- **2 pairs of forcep**

Before the adult wing dissection, the flies are collected from bottles in which they were breed. Each bottle is labelled with the genotype of the flies it contains for example ***dsh GPF 5.7a /TM6b*** meaning flies with the dishevelled mutation tagged with GFP (green fluorescence protein).

All the flies in a bottle are emptied on to the surface of the extension of a binocular microscope. There is a tube leading from the extension which is connected to a $CO_2$ tank. $CO_2$ is directed to the extension; as $CO_2$ is denser than air it forms a film of $CO_2$ gas in the extension which holds the flies. This acts as a general anaesthetic thus knocking out the flies. Whilst the flies are immobile a paint brush can be used to gently separate the flies desired. To dissect the wings, one pair of forceps is used to hold the body of the fly while another is used to pull the wing from the body at the notch as indicated in the diagram below:

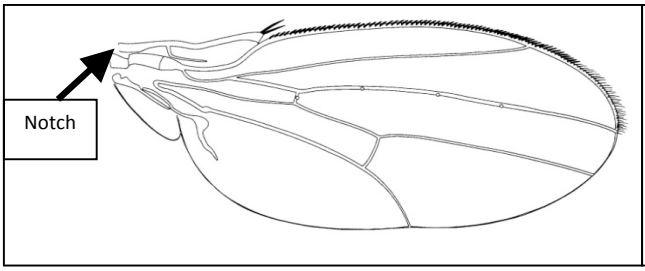


FIGURE 2: Diagram showing the point at which the wing is dissected from the drosophila body.

The wing is then placed on a slide. After 9/10 wings have been dissected, a fixative is dropped onto the slide. The slip cover is placed carefully over the slide; making sure not to displace the wings so that they do not lie on top of each other. A powerful light microscope is used to view the wings and a computer programme *ProGres* is used to take the picture used for analysis. The dissected flies are then disposed of in vegetable oil.

**Below are examples of wings I photographed:**

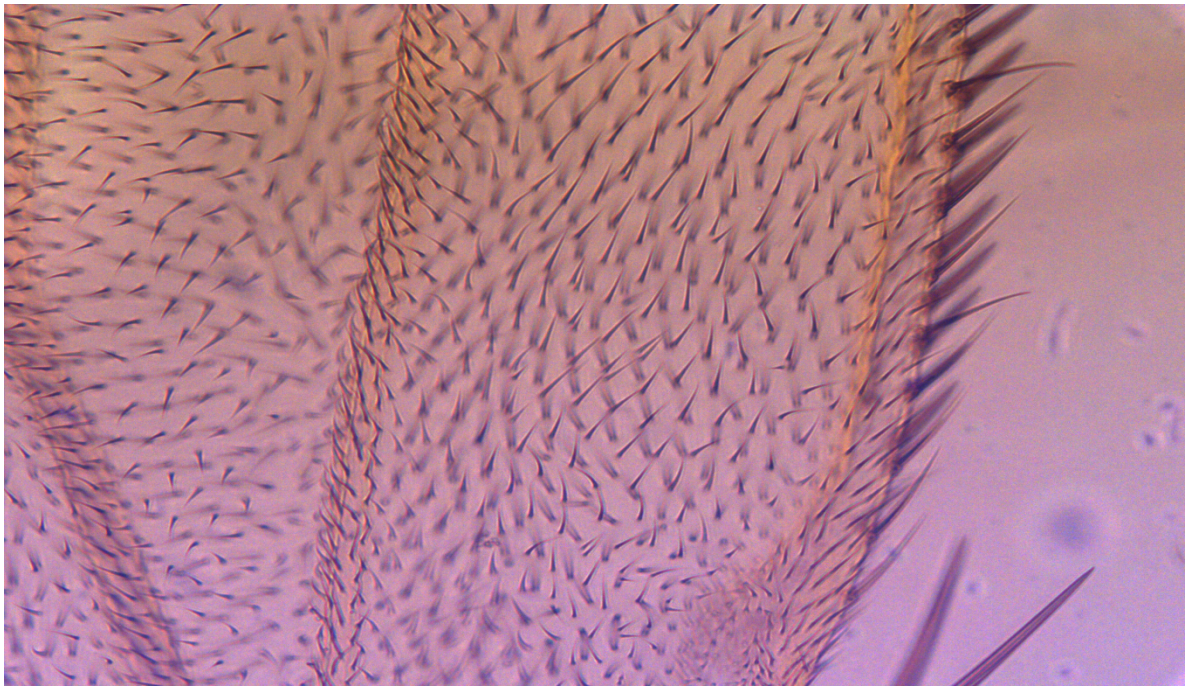

**Figure 4: picture of the wing of a dishevelled mutant**

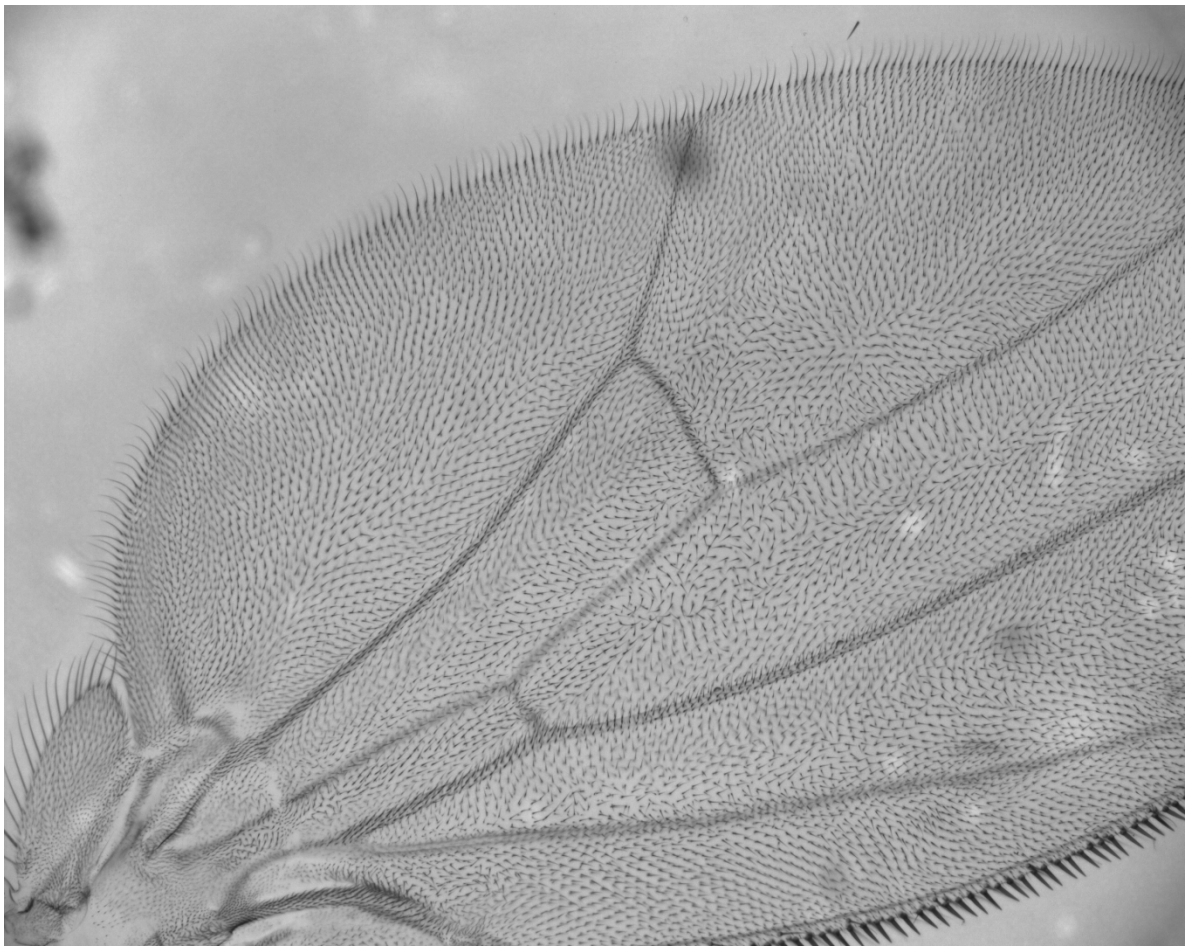

**Figure 5: picture of the wing of a dishevelled mutant**

## Pupal wing: for Immunofluorescence imaging

During the pupal stage of development, a number of important biological and chemical event occur; Immunofluorescence imaging looks to record these events.

## This is a brief overview of the lifecycle of *Drosophila melanogaster*

- ***Embryo/egg***: A mated female is capable of depositing up to 100 eggs per day, which she lays on or in the food. The egg develops for 24 hours.
- ***Larva*:** the egg develops into a 1st instar larva which immediately begins to feed on the medium. The next phase is a 2nd instar larva, the final being a 3rd instar larva. Both the 2nd and 3rd instar larvae eat through and into the medium where they remain for 4 to 5 days. At the end of this period, they emerge from the food and relocate onto the side of the bottle where they undergo pupariation.
- ***Pupa:*** A larval cuticle forms around the larva and develops into a pupal case. The larva goes from a milky tone to a darker tanned one. During this stage, which lasts about 4-5 days, metamorphosis occurs.
- ***Adult*:** on day 12 an adult fly emerges, inflates it wings and loses excess fluid.

## Collection of pre-pupa

Before the dissection, pre-pupa were collected from bottles in which the flies were breed. Each bottle is labelled with the genotype of the flies it contains for example ***dsh GPF 5.7a /TM6b*** meaning flies with the dishevelled mutation tagged with GFP (green fluorescence protein). The pre-pupal stage is just before the pupal stage and lasts for approximately 1 hour ; this is done so that the pupae can be aged approximately, give or take an hour. The bottles holding drosophila with the desired genotype are chosen and pre-pupa are gently extracted by using forceps to lightly lift then off the inner surface of the bottle. The pre-pupae are stored and aged for 48 hours at 18°C; during which they become pupa.

## [2] The Apparatus:

1. **Fixative: 8% paraformaldehyde** (PFA) in phosphate buffered saline (PBS). This can be freshly prepared or made from a stock solution.
2. **Phosphate buffered saline** (PBS)
3. **Washing PBT:PBT/PBT 0.01% TritonX-100PBS + 5% normal goat serum**
4. **Blocking solution** PBTN: PBTN/PBTN 0.01% TritonX-100/PBS
5. **ProLong gold antifade mounting medium**
6. **Microwell minitrays with lids**- 60 wells, low profile
7. **P1000 pipet**

[2] Taken from: Anne-Kathrin Classen1 et al. (2007). **Imaging Drosophila Pupal Wing Morphogenesis**. In: Christian Dahmann. ***Drosophila: Methods and Protocols***. Germany: Springer Protocols. 265-275.

8. 2 **pairs of forceps**
9. **Round petri dish lids** (used for dissection)
10. **Watch makers glass** with PBT
11. **Pipettes** (100-200µl)
12. **Wet paintbrush**
13. **Paper towel**

## [3]The procedure:

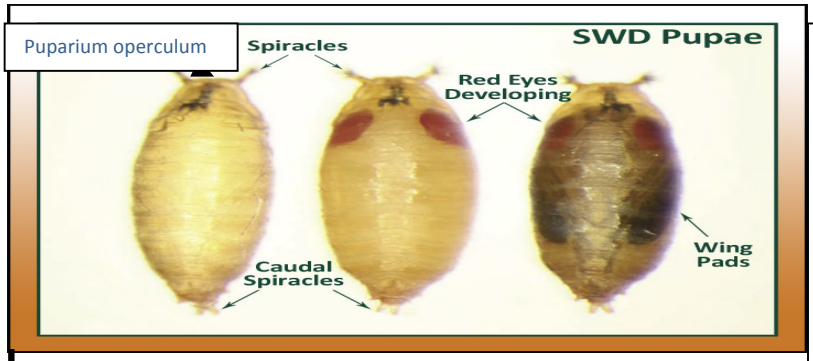


FIGURE 6: A diagram showing a labelled pupa of the drosophila fly.

Obtained from :
http://www.google.co.uk/imgres?q=drosohila+pupa&um=1&hl=en&qscrl=1&nord=1&rlz=1T4DKUK_en-GBGB271GB272&tbm=isch&tbnid=rhLhArBgGArmeM:&imgrefurl=http://whatcom.wsu.edu/ipm/swd/slides.html&docid=DkQt8Ev7xC2tYM&w=727&h=562&ei=YZRzTry4F42p8QPmxMzUDQ&zoom=1&iact=hc&vpx=761&vpy=100&dur=765&hovh=197&hovw=255&tx=167&ty=107&page=2&tbnh=106&tbnw=137&start=14&ndsp=12&ved=1t:429,r:5,s:14&biw=1054&bih=458

1. Pipette 8 40µl drops of the fixative onto petri dish lid.
2. Remove the pupae from the test tube with a wet paintbrush. Place a pupa in each droplet submerging it
3. Using one pair of the forceps, carefully grab the caudal spiracles to hold the pupa down.
4. Grab the Puparium operculum with the second pair of forceps and gently remove it to expose the head. Using the forceps, gently poke a small hole in the head. To increase the hole, move the forceps a little further into the head and open slightly. Poke a small hole in the cuticle on each wing.
5. Leave this pupa and move on to the next, this will allow time for the fixative to enter the opening
6. Repeat steps 1-5 until the last pupa
7. Go back to the first pupa and grab the caudal spiracles to hold the pupa down again. Using the forceps remove some abdominal tissue and wipe on the paper towel. Then, still holding the caudal spiracles, grab the ventral thoracic cuticle and pull the pupa out of the pupal case.
8. Remove the cuticle and discard of it on the paper towel

[3] Adapted from: Anne-Kathrin Classen1 et al. (2007). **Imaging Drosophila Pupal Wing Morphogenesis**. In: Christian Dahmann. ***Drosophila: Methods and Protocols***. Germany: Springer Protocols. 265-275.

9. Use a pair of forceps to pin the pupa down at the abdomen. Carefully tease the wing from the body of the fly. Remove the pupal wing at the notch.
10. Pick the wing up with a droplet placed in between the pair of forceps and place it on a watch maker's glass with PBT. Wash the pupal wings with a P1000 pipet.

I was unable to get a pupal wing. The pupae were extremely delicate and the debris of white fat that surround them made it difficult to distinguish the wings.

## Computational analysis

The project, still on going, is a PhD research venture by Qian Gao to model the phenomenon of Planar cell polarity in the wing of *Drosophila melanogaster* using hierarchically coloured petri nets (HCPN). Specifically this project is to model the signal transduction generated by the PCP module proteins; an explanation of the biological event of PCP can be found at the beginning of this Placement summary. The hexagonally shaped cell of the drosophila wings was represented by hexagonal cells divergently divided into specific sections to allow the mimicry of PCP by a computational model.

The main challenge posed by this project is that of modelling the interaction of the module proteins over the whole wing tissue. Currently, the modelling is focused on representing PCP in a patch of cells. Hierarchically coloured Petri nets are used to mathematically and visually simulated the action of PCP module proteins

I learnt the basics of snoopy i.e. how to use the character to create simple petri net for example:

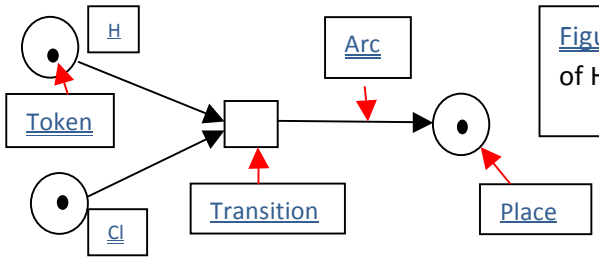


Figure 5: This reaction is the formation of Hydrochloric acid

I attempted to construct a petri net for the cell cycle. I realised that very specific information is needed to build functioning petri nets. I was given the opportunity to proofread the English translation of the

I think modelling is a powerful tool that can be used in research. It present information in a way that is visible and mobile thus easier to understand. It can help to study or predict the

behaviour of systems in biology and the other sciences. I think it also has the potential to be used for educational purposes, catering to the need for visual learning aids.

## Personal experience summary

I imaged hair orientation on the wings of drosophila for the study of Planar cell polarity via a number of procedures. I was assigned the tasks of culturing my own line of drosophila. To do this I; prepared the food medium, selected flies for breeding, monitored their growth and development. The main procedures I carried out were dissection of wings from Adult and Pupal specimens. The appropriate samples were mounted on slides for viewing under a microscope. The purpose of this was to image the hair patterns in different mutants of PCP genes.

This part of the placement developed my confidence, time management, communication, learning ability and prioritisation skills. I had to organise and plan for the week ahead of time, this taught me to gather and organise tasks efficiently. As I wrote an article on Planar cell polarity, I developed my ability to gather, organise, understand and present information. Giving a talk at the tutorial in Heidelberg Germany also strengthened my ability to convey information clearly and accurately.

As a part of my placement, I also attended meetings with the team of individuals mentioned on the first page. This gave me an insight as to how research projects work and how different people contribute to it.

During this placement I also learnt a lot; about myself, Planar cell polarity and scientific research. Research is the meshing of minds to create, improve and discover. It is necessary to be creative and flexible when researching as things do not always go according to plan. The placement taught me how to react to new situations, convey my thoughts and motivate myself to finish a task. I have also gained a lot of confidence in my abilities to; accomplish a goal, to learn new topics quickly and work under pressure. I most enjoyed giving a talk at the tutorial in Heidelberg, meeting and interacting with such a diverse group of people.

Overall this was a good placement. It exposed me to a wide range of experiences and challenged me. My skills in presenting and scientific writing have vastly improved. All the skills I mentioned are essential for the workplace.

**Acknowledgements:**

I would like to thank **Professor David Gilbert, Professor David Tree, Professor Monika Heiner, Ms Qian Gao, Mr Daniele Maccagnola** and **Mr Ville Rantanen** for this experience**.**