Shape and venation of wings in the presence of a gain of function allele of *Egfr*

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10 eininga ritgerð sem er hluti af
Baccalaureus Scientiarum gráðu í líffræði

Leiðbeinandi
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Líf- og umhverfisvísindadeild
Verkfræði- og náttúruvísindasvið
Háskóli Íslands
Reykjavík, Janúar 2010

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Genetics of ectopic veins
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Abstract

Threshold traits are responsible for a number of inheritable phenotypic characteristics in organisms, yet their nature remains elusive and they have proven hard to study. Here we show the wing shape of *Drosophila melanogaster*, genetic perturbations and morphometic analysis can be used to investigate the genetic and developmental underpinnings of threshold characters. The E1 allele of the *Egfr* gene was used to study the genetic and developmental aspect of threshold characters. An association between a mutation in the *Egfr* promoter and the relative placement of the two crossveins was also re-evaluated. More than a hundred inbred strains were crossed to two strains with the same *Egfr-E1* allele; strain 5144 and strain 1564. Results showed that the extra veins form in specific parts of the wing, not randomly all over and are seemingly independently of extra veins in other parts of the wing. Results also showed that cross allowed for the presence of the extra vein, and sex seemed to determine both its presence and length. The association between the *Egfr* polymorphism and the C region was confirmed. An association between the *Egfr* polymorphism and the formation of extra veins was found, however this depends on the genetic background and the sex of the individual. It was determined that the same regulatory SNP affects the continuous trait and the manifestation of a threshold trait and implicates the role of *Egfr* signalling in both placement of veins, and also in the formation of the vein material.

For my mom and dad
Rebekka and Godson

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Introduction

Threshold traits

Some traits that express two or a few phenotypic characteristics have polygenic inheritance. The phenotypic expressions of these traits do not follow the simple Mendelian pattern of inheritance, that is their phenotypic characteristic is either present or absent for example in disease susceptibility where the individual could be infected or not infected by a disease. These traits although they seem not to be quantitative traits, after examination have been found out to be inherited in much the same way as quantitative traits in a continuous manner. These characters have what is called a "threshold" on an underlying trait called the "liability" which determines the expression or non-expression of the trait. In a simple model the liability trait is assumed to be normally distributed and individuals below the threshold point express one type form and individuals above the threshold point exhibit another character value, like healthy vs. sick (Figure 1.1). An example can be seen with birthing in cows where they have either one or twin births at a time. The number of births is determined by an underlying variable, the level of circulating gonadotropic hormones. This hormone determines the number of eggs that is shed at a time and the intra-uterine factors which affect embryonic survival and determines monozygotic twining. Here the level of the circulating gonadotropic hormone follows a continuous distribution, however if a cow has levels above its so called threshold point then the cow births twin calves, but if it is below then it births one calf which follows a metric distribution (Falconer & Mackay 1996).

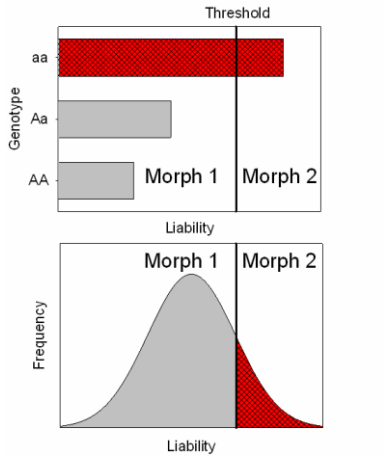

Figure 1.1: An illustration of the threshold model showing a single gene with two alleles (top) and its polygenic inheritance (bottom). (Roff 2008)

Threshold traits are not only influenced by genes but also by the environment and chance. In Figure 1.1 above the threshold model shows one underlying trait resulting in a dimorphism. An example is sex ratio in reptiles where the sex is determined by the genes and the temperature of the environment. Other examples include the risk of heart attack and diabetes, cyclomorphosis, paedomorphosis in salamanders, wing dimorphism

in insects (Roff 1998), and in wing the presence or absence of extra veins as is being observed here. There can be more than one underlying trait interacting with each other and having a threshold point which for instance results in the dimorphism (Figure 1.2a). There can also be more than two phenotypic characters, which results from having two threshold points (Figure 1.2b). An example is having single, twin or triplet births in humans, cows or sheep (Falconer & Mackay 1996).

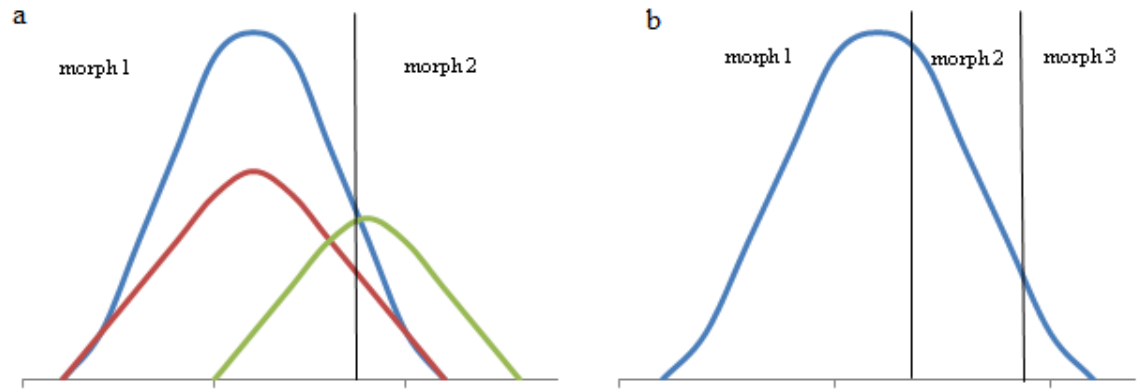

Figure 1.2: Threshold models. (a) A threshold trait having 2 phenotypic characters resulting from three underlying traits interacting and having a single threshold point. (b) A threshold trait having three phenotypic characters resulting from two threshold points.

Wing shape

Wing shape in *Drosophila* is a complex morphological trait with a developmental process that is also complicated. In embryogenesis, the wing develops from a group of cells (~ 15-20) that invaginate from the lateral ectoderm of the mesothoracic segment to form the wing disc (Crozatier *et al*. 2004). This wing disc is divided into the anterior (A) and posterior (P) compartment cells. The protein *Engrailed* (*En*) initiates the A-P patterning in the wing and patterns the posterior region of the wing imaginal disc (Bier 2000). *En* also activated the short range paracrine signalling ligand *Hedgehog* (*Hh*) at the boundary between the anterior and posterior territories. *Hh* from the P cells is secreted and causes the expression of *Decepentaplegic* (*Dpp*), which is a ligand of the *TGF-β* signalling pathway (same). *Dpp* has a long range effect and controls the growth and A-P patterning of the wing disc (Crozatier *et al*. 2004). The veins and crossveins are formed by the regulation of *egfr* signalling and are maintained by the *TGF-β* pathway (Dworkin & Gibson 2006).

Wing shapes is determined by the length and placement of wing veins. The wing veins consist of 5 longitudinal veins (L1-L5) and 2 crossveins (CV1 and CV2 - see Figure 1.4). The patterning of the veins is directed by the *Hh* and *Dpp* signalling pathways (de Celis & Diaz-Benjumea 2003). *Hh* has a short range effect and is mediated by the transcription factors *Collier* (*Col*) or *Knot* (*Kn*) in the A-P cells. *Col* or *Kn* upregulates *D-SRF* and downregulates *Epidermal growth factor receptor (Egfr)* in the cells there causing the formation of the L3-L4 cross vein (Figure 1.3). *Col* or *Kn* also upregulates the expression of the *Egfr* ligand *Vein* (*Vn*), which activates the Egfr pathway, and downregulates *Dpp* signalling by the transcriptional repression of *Thickveins* (*Tkv*) allowing for the formation of the L4 vein (Figure 1.3). *Hh* and *Dpp* signalling controls

the expression and activity of *Iroquois* (*iro*) and causes the formation of the L3 vein (Figure 1.3). The expression of *iro* by the *Hh* signalling is modulated by the repressing activity of *Spalt* (*Sal*) and *Spalt-related* (*Salr*). The L2 and L5 veins are formed by *Dpp* signalling through regulation of *Sal* and *Salr*, and *optomotorblind* (*omb*) respectively. *Sal* and *Salr* regulates the expression of the gene complex *Knirps* (*Kni*) and *Knirps-related* (*Knrl*) and *iro*. The L2 vein is formed by the repressing activity of *Kni* and *Knrl* on D-SRF which then activates the expression of *rhomboid* (*rho*) in some cells marking out the position of the L2 vein (Figure 1.3). *Omb* activates *abruptex* (*ab*) in some cells causing the formation of the L5 vein (Crozatier *et al*. 2004). The pro-veins are first formed by the expression of these genes above, then at the border and centre of each pro-vein, *Notch* and *Egfr* signalling controls the thickness of the veins (de Celis & Diaz-Benjumea 2003).

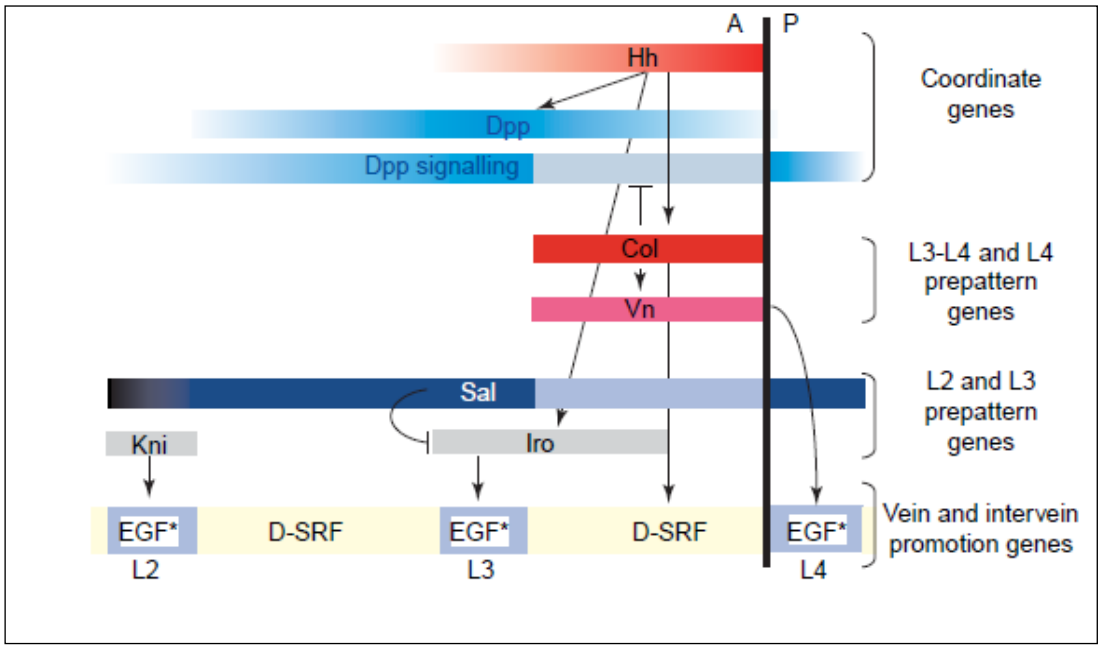

Figure 1.3: Expression pattern of the coordinate, prepattern and vein- and crossvein-promting genes that are involved in the formation and positioning of the L2, L3 and L4 vein in the wing. The potential vein and crossvein territories are shaded blue and yellow respectively. The domains of individual gene expression are represented by the coloured horizontal bars. The straight arrows and crossed lines show director indirect transcriptional activation and repression respectively (Crozatier et al. 2004).

Wing shape is highly heritable (Birdall *et al*. 2000) and under strict genetic control (see above). The shape seems to be determined by the placement and length of the veins (Palson and Gibson 2004), or at least factors that also dictate the placement of veins and junctions of veins/crossveins and veins/margin. It has been shown that there is an association between one mutation in the *Egfr* promoter (T30200C) and wing shape (Palsson and Gibson 2004). This association was with the shape of the central region (C) (Figure 1.4). Specifically the T30200C associates with a decrease in the spacing between the anterior and posterior cross vein and was consistent with a slight loss of function of the Egfr signaling (Palsson *et al*. 2005). This association held in samples

flies from North Carolina and Kenya, and even in wild caught flies from NC (Dworkin *et al.* 2005). Here I will reevaluate this association in with our dataset, and also plan to test whether the T30200C polymorphism affects extra vein formation in the wing.

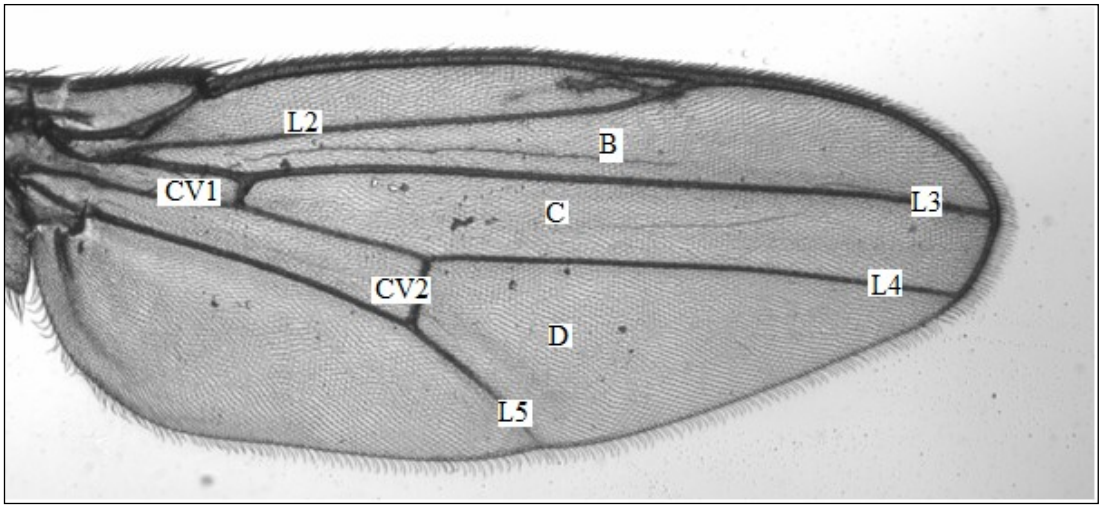

Figure 1.4: Figure of the Drosophila wing showing the B, C and D regions, the L2, L3, L4, L5 longitudinal veins and crossveins CV1 and CV2.

Egfr mutations – the E1 allele

The *Epidermal growth factor receptor* (*Egfr*) is a pleiotropic gene involved in a number of cell processes including eye development, cell division, cell fate decisions and differentiation and in wing development and venation (Palsson and Gibson 2003, Klambt 2000, Crozatier *et al.* 2004). Its activity is precisely regulated probably due to the number of cellular functions it is involved in. Four ligands are known to bind to Egfr, encoded by are *Vein, Gurken* and *Spitz* are activating ligands, and *Argos* encodes an inhibitory ligand (Klambt 2000). Two types of transcripts are encoded by the *Egfr* gene, leading to two isoforms of the receptor, and during development *Egfr* is expressed differently and in different amounts at different stages (Lev *et al*. 1985). *Egfr* is required for wing vein differentiation and its hyperactivity leads to abnormal positioning in vein differentiation (Lesokhin *et al*. 1999). Developmental genetic most often rely on *loss-of-function* alleles, but mutations altering function or that lead to ectopic expression of genes (*gain of function* mutations) are also extremely informative (Griffiths *et al*. 2008). *Egfr* affects different processes in separate tissues during development and a loss or gain of function mutation leads to different responses in the gene function (Clifford & Schupbach 1994).

The best known *gain-of-function* allele of *Egfr* is the *ellipse* allele. *Ellipse* (*E1*) mutations have an effect on the formation of the ommatidium during eye development (Lesokhin *et al*. 1999). The mutation is dominant, and suppresses the ommatidium development resulting in a reduction of the eye size (Lesokhin *et al*. 1999). The gain of function of the *E1* allele increases the average number of photoreceptor cells in each ommatidium during eye development leading to an eye roughness phenotype. It depends on the genetic background how much eye roughness is observed when E1 is

crossed to different inbred lines. That demonstrates a genetic variation for this trait. As normal eye does not exhibit roughness, one can say that this is hidden cryptic genetic variation (Dworkin *et al*. 2003), see Gibson and Dworkin (2004) for a review. Analysis by Dworkin *et al.* (2003) revealed that there is also an association between *Egfr* polymorphisms and variation in eye roughness. Dworkin *et al.* (2003) used the gain of function of the allele *E1* to perturb eye development and uncover hidden genetic variation. One way of studying this is to change the environment and another is to change the genetics. Here we follow the approach of Dworkin *et al.* (2003) by using genetics to uncover hidden genetic variation. The E1 allele disrupts eye development in *Drosophila*, it disorganises the ommatidia, causes the eye to be irregularly shaped or fused and this gives the eye a rough appearance (Clifford & Schupbach 1989). Loss of *Egfr* function leads to gaps of varying sizes in the veins of the wings (Clifford & Schupbach 1989). It is known that the E1 allele causes extra vein material to form and here we want to document these effects more carefully.

Here we plan to use the approach of Dworkin *et al.* (2003), to induce extra vein materials in the wing with crosses to two E1 containing strains. By simultaneously evaluating extra veins and continuous variables (shape) we hope to gain insights into the nature of threshold traits. We also plan to evaluate whether the silent *Egfr* polymorphisms identified by Dworkin *et al.* (2003) are correlated with formation of extra vein material.

In this study we used the E1 allele of the *Egfr* gene to study the genetic and developmental aspect of threshold characters. An association between a mutation in the *EGFR* promoter and the relative placement of the two crossveins is also re-evaluated (Palsson and Gibson 2004). More than hundred inbred strains were crossed to two strains with the same *Egfr-E1* allele; strain 5144 and strain 1564. Strain 1564 contains a *narrow* allele in addition to the E1 allele, *narrow* causes elongated wings (Flybase 2010). My results show how wing shape, genetic perturbations and morphometic analysis can be used to investigate the genetic and developmental underpinnings of threshold characters.

Aims

I set out to investigate the relation between continuous variables and threshold traits (extra veins). The specific research questions are:

- Is there is any difference in extra vein formation between crosses (5144 and 1564)?
- Do extra veins correlate to the shape or size of the wing?
- Does cross, sex or genotype have an effect on the extra veins?
- Is there is an association between *Egfr* polymorphism C30200T and the shape of region C on the wing?
- Is there is an association between *Egfr* polymorphisms and the extra veins?

Materials and Methods

Capture of data

The wings were captured earlier, in junction with the studies of Dworkin *et al.* (2003). In short the flies where grown on standard cornmeal medium at constant temperature (25°C) in a humidity controlled incubator. Crosses were done using the *Egfr* allele *Ellipse*^{1} (*Egfr*^{E1}, A887T, Dominguez *et al*. 1998). Two crosses containing the *Egfr*^{E} allele was used (BL-1564 and BL-5144) and a panel of 101 inbred lines from North Carolina (Dworkin *et al*. 2003, and Palsson and Gibson 2004). Five males of one stock were mated with 5 virgin females from an inbred line, they were allowed to lay eggs for 3 days and then the parents were discarded. As the *E1* allele carrying chromosomes were kept over a Curly bearing balancer then we could identify the *E1/wt* heterozygous individuals as those with straight wings. For each cross, sex and line combination we harvested around 10 individuals (on occasion they were as few as 6), from two replicate vials put up on separate dates.

The wings were removed with microscissors and mounted on a slide, kept in place with a microslip taped to the slide. The wings were photographed and using a SPOT camera attached to a Nikon Eclipse microscope at low magnification, and stored as TIFF files for further analysis (see Palsson and Gibson 2000). This work was completed by Arnar Palsson, Kelli Birdsall and James Dodgson in 2002).

Processing of landmarks

I used the Scion imaging program to record the digitized image of the shape and extra veins from the wings. The picture images of the wings were loaded into the Scion imaging program (www.scioncorp.com). The X and Y coordinate for 9 landmarks and the length of extra veins were estimated by XY on either end when present (Figure 2.1). The data obtained was processed in Excel to calculate the lengths (distance) of the extra veins and the area of the B, C and D regions (Figure 2.1) along with their total area with simple geometric formulas.

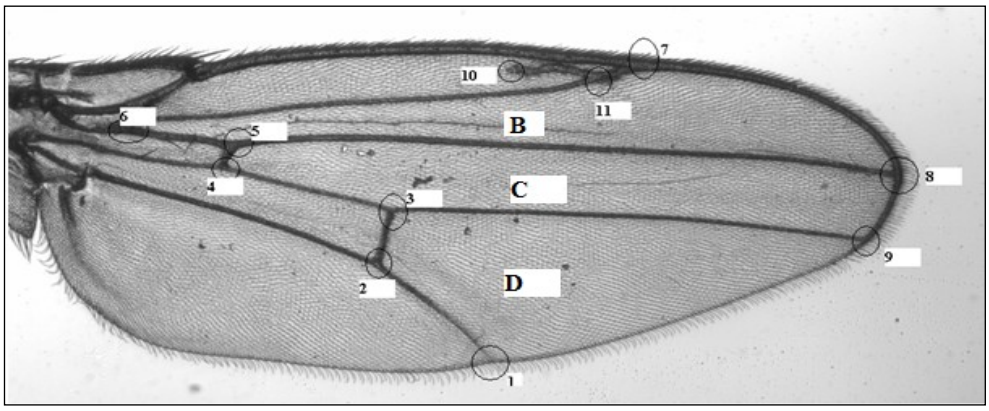

Figure 2.1: Figure of the wing showing the X-Y coordinates of the landmarks that where measured (1 -11). B, C and D are the different regions of the wing. Points 5, 6, 7 and 8 make up the B region. Points 3, 4, 5, 8 and 9 make up the C region. Points 1, 2, 3 and 9 make up the D region.

$$Distance = \sqrt{[(X_1 - X_2)^2 + (Y_1 - Y_2)^2]}$$

$$Area = \frac{\{[(X_1 \cdot Y_2) - (X_2 \cdot Y_1)] + [(X_2 \cdot Y_3) - (X_3 \cdot Y_2)] + [(X_3 \cdot Y_1) - (X_1 \cdot Y_3)]\}}{2}$$

$$T\ area = B\ area + C\ area + D\ area$$

We first inspected 1000 wings from the data set to determine where extra veins tended to form, and found 5 locations where they were most likely to appear. We looked specifically for deltas at the junctions of L3and the margin and L4 and the margin but found none. During analysis of the total set (6325) we were also alert to other extra vein material, but none was found at comparable frequency to the five regions we discuss here. The 5 extra venations that where measured where labelled evA, evB, evC1, evC2, and evD. However, not all extra veins could be found on each of the wings. Figure 2.2 shows the extra veins that where found and recorded. We expected to find evC3 extra veins where vein L4 meets the margin (so called delta), but none was found in the samples (see appendix A).

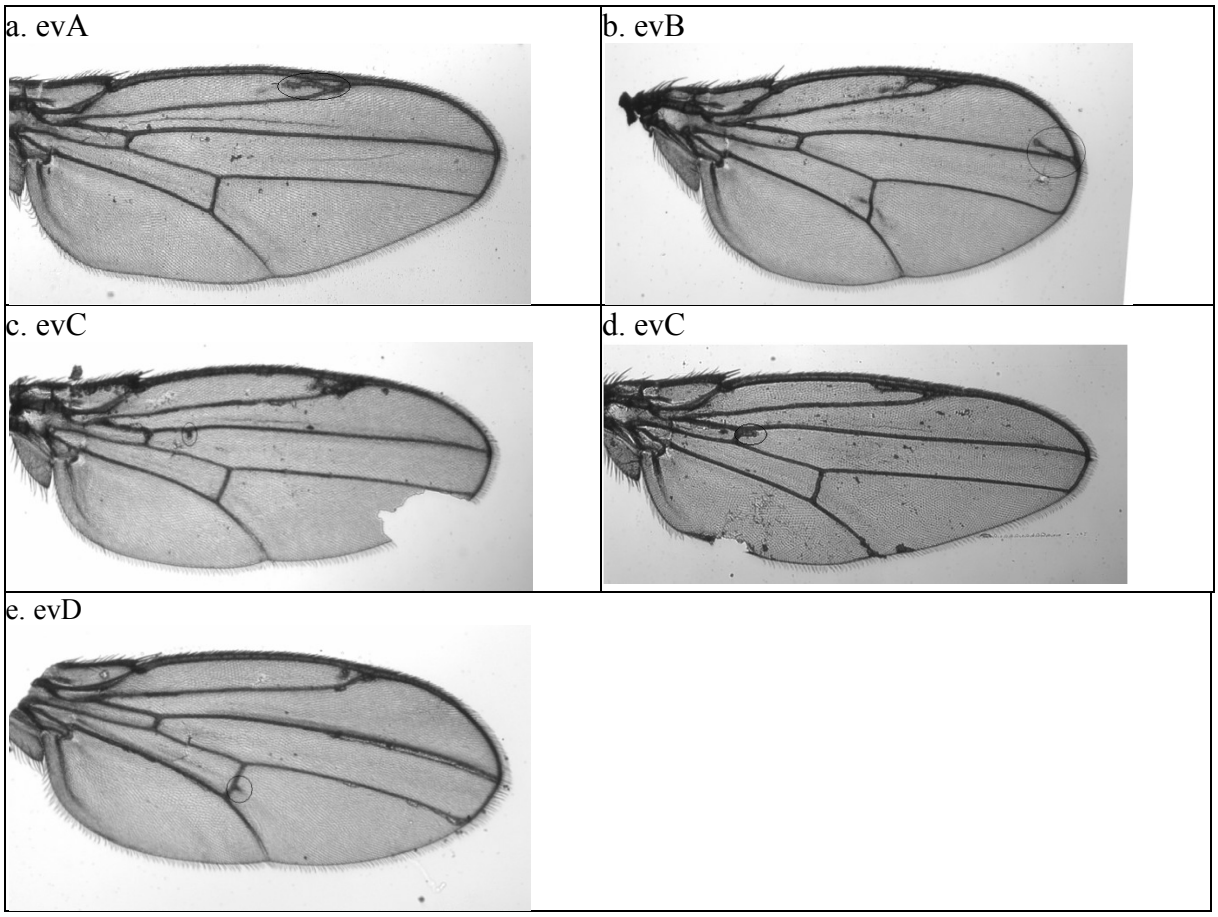

Figure 2.2: Figures showing the extra veins that were found in the wing samples. (a) Wing showing the W extra vein that was recorded as evA. (b) Wing showing the evB extra vein. (c) Wing showing the evC1 extra vein. (d) Wing showing the evC2 extra vein. (e) Wing showing the evD extra vein. Note the notched margins in specimens C and D are due to handling of wings, those are not biological phenomena.

Morphometric analysis

In order to summarize the shape variation in the dataset I used the tps relw program (F. James Rohlf, http://life.bio.sunysb.edu/morph/). The X-Y coordinates obtained for the 9 landmarks where loaded into the tps relw program for quality check. If the image obtained is in the form of the wing, and no outliers were present, then we assume that no gross errors in measurements were made (Figure 2.3). The error rate due to missing or extra landmarks was pretty low about 1 in 6000 wings, those where easily corrected. Certainly there will be slight errors in placement of landmarks but we assume that those are random and will not bias the data set systematically.

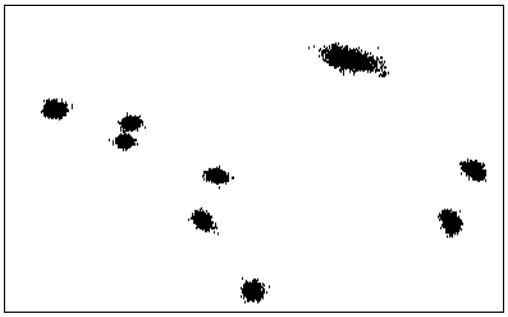

Figure 2.3: Image from the tps relw program showing the 9 landmarks from 2919 individuals of cross 5144 that were measured using the scion imaging program. Each dot represents the XY coordinate of one wing

The relative warp scores matrix for the whole wing (W), B, C and D regions were obtained from the tps relw program. The data for all the specimens were combined into Excel, and saved as a tab delimited file for analysis in R program (www.r-project.org).

Statistical analysis

The relative warp scores for the whole wing (W), B, C and D regions along with the distance measurements of evA, evB, evC1, evC2, and evD extra veins, and the areas of the whole wing, B, C and D regions, were put together into a table and saved as a tab delimited file for analysis on R program. The file was imported into the R program for statistical analysis:

Description of the variation in traits: Consensus coordinates for extreme shapes were saved in the tps relw program and imported into Excel for visualization. These figures were used to map the relative shape of the whole wing, the B area, C area and D area. The data was split into 4 groups according to the cross and sex (m5144, f5144, m1564 and f1564). The means, standard deviation and median of the extra veins were then calculated for the groups. Histograms of the whole dataset were also drawn to visualize the distribution of the traits across the dataset.

The correlation between variables: In order to evaluate relationships between variables we calculated parametric (Pearson's) and non-parametric correlation (Spearman's) between the distances, areas and relative warp scores (cor() in R was used for both). This was done for the whole dataset and then for the data split by then split by cross and

sex (m5144, f5144, m1564 and f1564). The results were then exported into an excel file for visualization.

Test for effects of cross, sex, size, genotypes (lines) on the extra veins: We also investigated the relation between shape and extra veins - in order to try to uncover common axes of variation to the continuous and threshold characters. These analysis where done with linear models - lm() in R, generalized linear models - glm() and generalized additive models - gam() in R.

Test for association between Egfr *polymorphism and shape of region C*: verify results of Palsson and Gibson (2004).

Those were evaluated with linear model, for each cross separately. We ran a linear model with average values for C1 for each sex, cross and line combination, with the R function lm().

C1 = Genotype + Sex + G X S + error

Where Genotype indicates the status of T30200C (two alleles T or C) in *Egfr,* Sex is self explanatory and G X S investigates whether the impact of one variable on the trait depends on the other variable. This was calculated separately for C1 derived from the entire dataset and for C1 from the dataset split by cross, because the cross had really profound effects on shape and distorted the main axes of variation. In order to robustly evaluate the model we implemented a permutation of the data set (5000 times) and recalculated the F statistics for the overall model, on datasets subdivided by sex.

Test for association between Egfr *polymorphism and extra veins*: This is done statitically analytically to number 4 above. We evaluated the contribution of T30200C and 4 *Egfr* polymorphisms to variation in extra vein characters evA, evB and evC1 using similar models as described for re-evaluation of the C1-T30200C association.

Results

Description of variation in traits

The question asked here was if there was any difference in the frequency and amount of extra veins between the two crosses. To determine this, histograms were drawn, consensus pictures were visualized and the consensus coordinates were mapped in Excel, and the mean, standard deviation and median were also calculated and compared between the two crosses. The whole population shows a normal distribution for some of the traits. The size of the total area of the wing, the B, C and D area, all show a normal bell shaped distribution across the population (see appendix A, Figures 1 - 4). On the other hand, the lengths of the extra veins are not normally distributed. The extra vein evA is the only trait with a bell shaped distribution and rather few (~500 individuals out of more than 6000) do not have this extra vein (see appendix A, Figures 5 - 9). The other extra veins do not show this type of distribution, majority of specimens do not have any extra veins (except for in region A). Alternatively, very few wings have these extra veins in other regions.

Visually, there is a clear difference between the cross 5144 and 1564. Wings in cross 5144 are short and broad while the wings in cross 1564 are longer and thinner (Figure 3.1a – e and Figure 3.2a). Also, the shape of the different areas (B, C, and D) of the wing shows that those from cross 5144 are much shorter and broader than those from cross 1564, which are much longer and thinner (Figure 3.2a - d). This difference is obvious from the images in Figures 3.1a and b and from the recorded XY coordinates (Figure 3.1c vs. d). Note specifically the superimposed XY coordinates in figure 3.1e

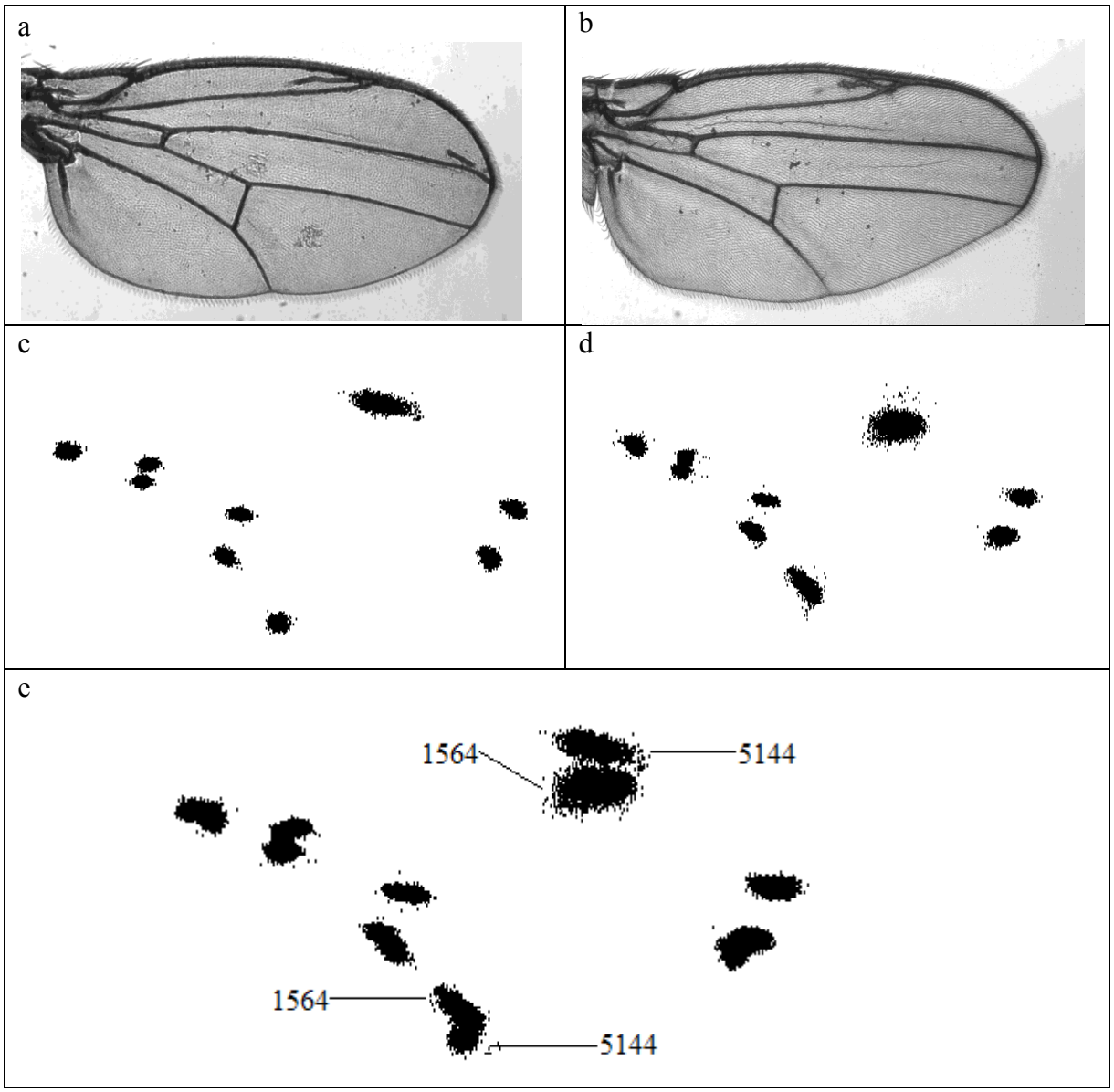

Figure 3.1: Figures showing the wing shape differences in cross 5144 and 1564. (a) Picture of the wing of cross 5144 replicate C. (b) Picture of the wing of cross 1564 replicate C. (c) Superimposed image of the wing shape of all 2919 individuals from cross 5144 from the tsp relw program (d) Superimposed image of the wing shape of all 3406 individuals from cross 1564 (e) Superimposed image of cross 5144 and cross 1564. We see how the landmarks are not on top of each other for both crosses.

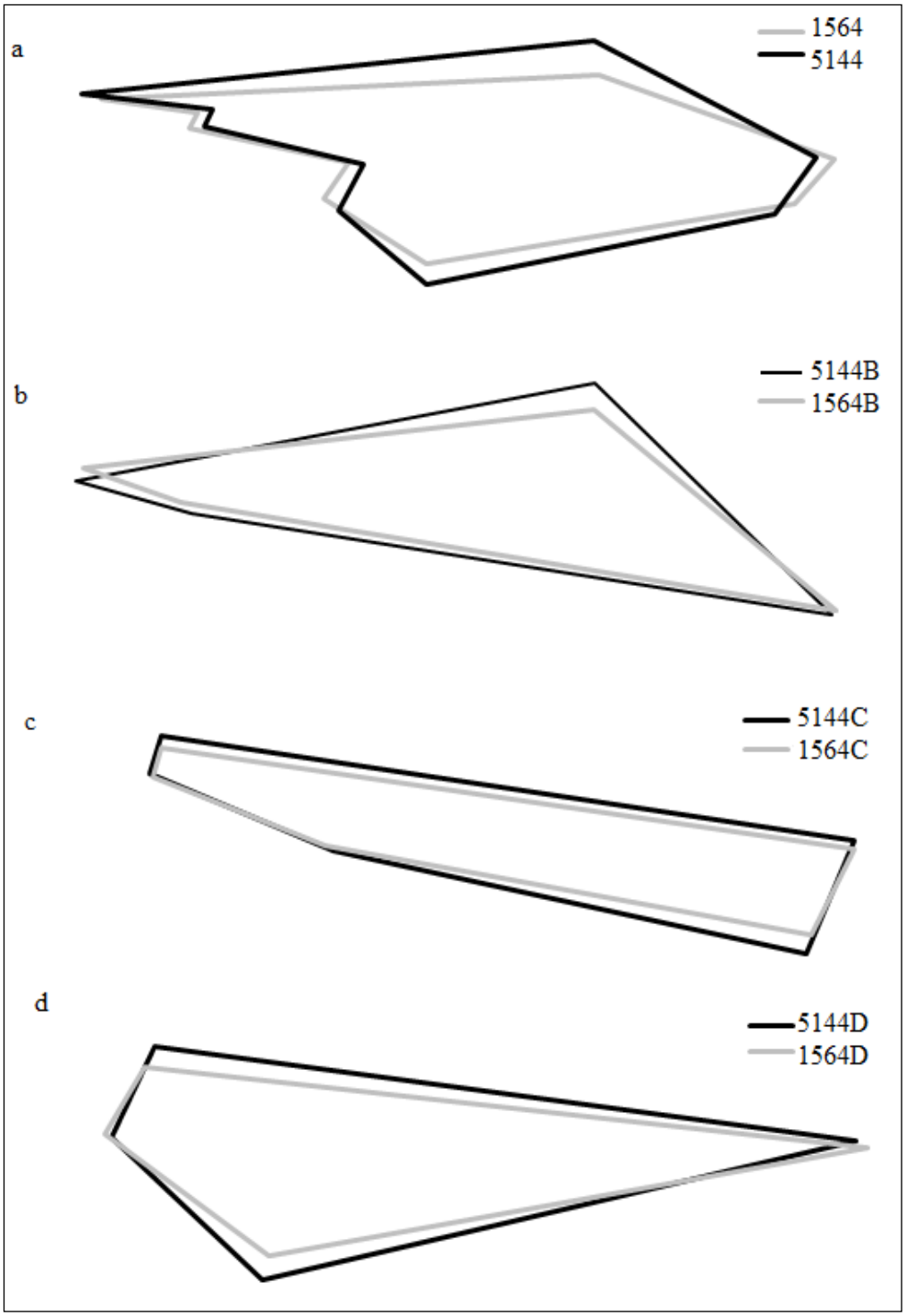

Figure 3.2: Figures showing the wing shape differences in cross 5144 and 1564. (a) Relative shape of the whole wing of cross 5144 and 1564, superimposed on one another. (b) Relative shape of the B area of the wing of both crosses. (c) Relative shape of the C area of the wing of both crosses (d) Relative shape of the D area of the wing of both crosses.

With the presence of extra veins, it can be see that with the extra vein evA is very common among both crosses. However, on average the extra vein evA in cross 5144 are longer than in cross 1564 (table 3.1). Also, the females of both crosses has more of the extra vein present than the males (table 3.1). We see the same tendency for the extra vein evB with cross 5144 having more and the females from both crosses having more. The opposite is seen for the extra vein evC1 with cross 1564 having more and the females from it having more (table 3.1). With cross 5144, both sexes have about the same amount of the extra vein evC1 (table 3.1). The extra veins evC2 and evD are very rare, but their presence seems to depend on cross and sex. For example the extra vein evC2 was found only in the females of cross 1564 and evD is found mostly in the females of cross 1564 (table 3.1)

Table 3.1: Comparison of the means, standard deviations and medians of the length of the extra veins found in cross 5144 and 1564.

Extra vein	Cross	Sex	Mean	Std dev	Median
evA	5144	f	0,79722	0,35530	0,77647
		m	0,66095	0,28875	0,63246
	1564	f	0,68040	0,37611	0,63273
		m	0,55699	0,37099	0,52010
evB	5144	f	0,07255	0,27438	0
		m	0,04787	0,15997	0
	1564	f	0,00780	0,07292	0
		m	0,00103	0,02504	0
evC1	5144	f	0,00025	0,00685	0
		m	0,00028	0,00785	0
	1564	f	0,01264	0,05919	0
		m	0,00154	0,02181	0
evC2	5144	f	0	0	0
		m	0	0	0
	1564	f	0,00025	0,01025	0
		m	0	0	0
evD	5144	f	0	0	0
		m	0,00015	0,00559	0
	1564	f	0,00027	0,00790	0
		m	0,00008	0,00318	0

Correlations between extra veins and other variables

We wanted to determine (i) if the presence of one extra vein correlated with presence of other extra veins (if there where general extra vein tendencies in the entire wing blade) and (ii) if the extra veins correlated with the shape or size of the wing. A parametric (Pearson's) and non-parametric (Spearman's) correlation was calculated between the extra vein measures, area of the whole wing and specific regions and relative warp scores. The results showed no correlation among the extra veins in the different parts of the wing. This argues against a wing-wide signal that enhances vein formation in the entire wing. Regarding our second question we found correlation between evA and the

total area and B, C and D areas (Table 3.2, also see appendix B, table 1). Furthermore evB shows a strong correlation with the total area (r=0.10) and B area (r=0.13) (Table 3.2). The extra vein evC1 shows a weak correlation to the C area (r=0.08) and D area (r=0.09) (Table 3.2). With 6000 specimens this is extremely significant, even if the correlation coefficients are tiny. The non-parametric (Spearman) correlation resulted in similar results as the parametric correlation (see appendix B, table 2). For the males in cross 5144, there is no correlation among the extra veins, and the strongest correlation the extra vein evA shows is with the total area and size of the wing parts (measured by the areas) (see appendix B, table 3). For the females of cross 5144, there is no correlation among the extra veins and only the extra vein evA shows the strongest correlation with the total area and size of the wing parts (see appendix B, table 4). For the males of cross 1564, there is no correlation among the extra veins and only the extra vein evA shows the strongest correlation with the total area and size of the wing (see appendix B, table 5). For the females of cross 5144, only extra vein evA shows the strongest correlation with the total area and B area (see appendix B, table 6).

Table 3.2: Parametric (Pearson's) correlation matrix of the whole population showing the correlation between the extra veins with other variables. See appendix B table 1 for full data.

	evA	**evB**	**evC1**	**evC2**	**evD**
evA	1.00	0.04	0.01	0.01	0.00
evB	0.04	1.00	-0.01	0.00	0.00
evC1	0.01	-0.01	1.00	0.00	0.03
evC2	0.01	0.00	0.00	1.00	0.00
evD	0.00	0.00	0.03	0.00	1.00
Tarea	0.29	0.10	0.04	0.02	0.01
Barea	0.31	0.13	-0.04	0.02	0.01
Carea	0.20	0.07	0.08	0.01	0.00
Darea	0.21	0.03	0.09	0.02	0.01
W1	-0.16	-0.18	0.11	-0.01	0.01
W2	0.01	0.06	0.03	0.00	0.00
W3	0.06	0.02	-0.03	0.00	0.00
W4	0.14	0.03	0.06	0.00	0.00
W5	0.00	-0.05	-0.02	0.01	0.02
W6	-0.03	0.06	0.02	0.01	0.00
B1	-0.16	-0.15	0.11	-0.01	0.01
B2	-0.02	-0.07	-0.02	0.00	0.00
B3	-0.13	-0.05	-0.05	0.01	0.01
C1	-0.14	-0.18	0.08	-0.01	0.01
C2	0.00	-0.10	0.05	0.01	0.02
C3	-0.05	-0.04	-0.05	0.00	0.02
D1	0.13	0.16	-0.10	0.01	-0.01
D2	0.14	0.07	0.02	0.01	0.01
D3	0.01	-0.03	0.00	-0.01	0.02

* Key: Boxes in white represent 0.00 – 0.05 (no correlation)
Boxes in light gray represent 0.06 - 0.15 (weak correlation)
Boxes in gray represent 0.16-0.25 gray (strong correlation)
Boxes in dark gray represent 0.26 and larger (strongest correlation)

Test for effects of cross, sex, size, genotypes (lines) on the extra veins

We wanted to determine if cross, sex or genotype had any effect on the extra veins. We calculated the average length of the extra veins in the crosses of the 113 inbred lines and represented it on a graph. The results show that the cross, sex, and genotype affects the occurrence of extra veins and the amount of vein material especially in evA, evB and evC1 (figure 3.3). The graph shows that the extra vein evA occurs in both crosses; however, we see that the occurrences are slightly greater in the females of the population. The extra vein evB occurs mainly in cross 5144 and in the females, while the extra vein evC1 mainly occurs in cross 1564 and in females. Statistical support for this comes from linear models that were performed to test for the association between the polymorphism and the extra vein (see tables in appendix D). Sex seems to affect the length of the extra veins more strongly than the cross or the lines.

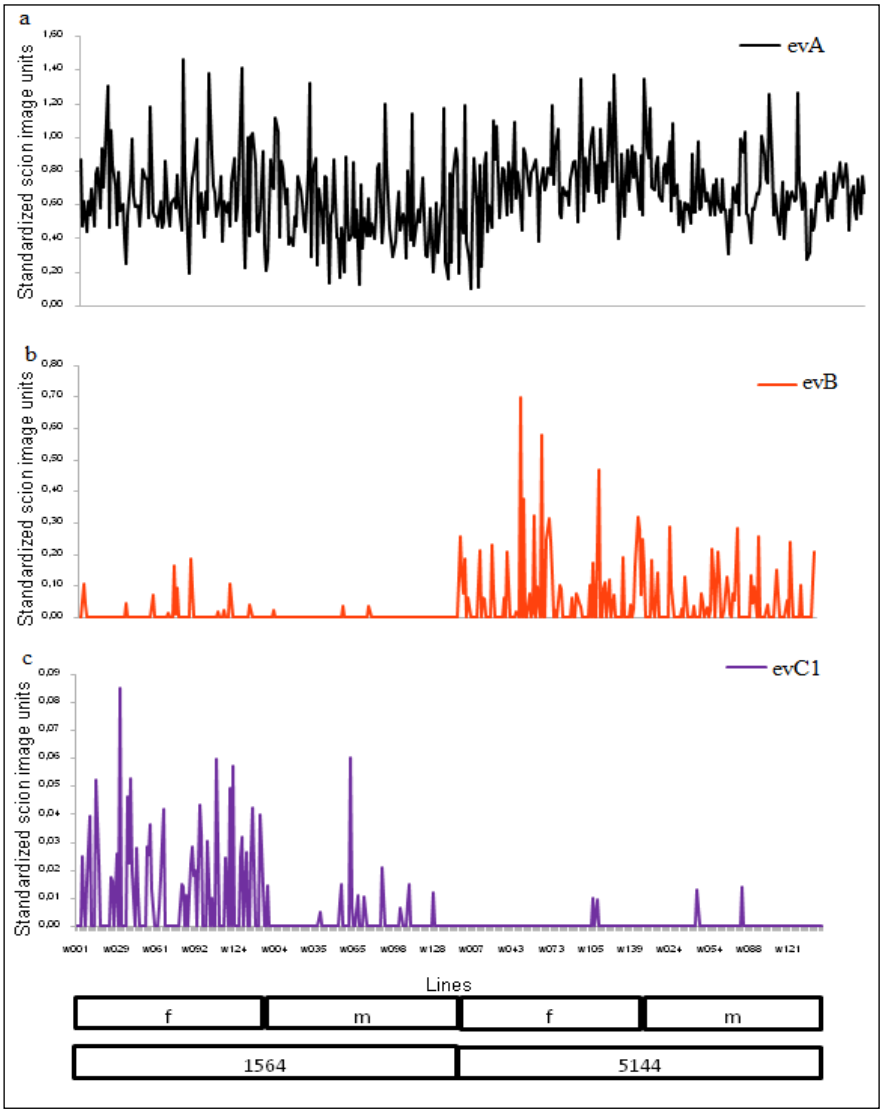

Figure 3.3: Average length of the extra veins evA, evB and evC1 for each combination of line, sex and cross (1564, 5144).

Test for association between *Egfr* polymorphism and shape of region C

We wanted to determine if there is an association between *Egfr* polymorphism C30200T and the shape of region C on the wing. This was evaluated with linear models, for each cross separately. The results showed a significant association between the shape of the first principal component for the C region with the C302000T SNP: p = 0,0127 in c5144 and p = 0,0013 in c1564 (Table 3.3). We also evaluated this by doing reduced models by sex and cross, see table 3.4. Recalculation the F statistics from 5000 permutation on datasets subdivided by sex also resulted in a significant association between the shape of the C region with the SNP (Table 3.4).

Table 3.3: Linear models for the association between the C302000T SNP, crosses and sexes.

Cross	Term	Estimate	SD	t	p-value	R^2	$F_{(df, df)}$	p-value
5144	Intercept	0.0007	0.0013	0.5410	0.5895	0.1469	$10.62_{(3, 185)}$	1.79E-006
	GTYP	-0.0037	0.0015	-2.5170	0.0127			
	SEX	0.0031	0.0018	1.6840	0.0939			
	G x S	0.0013	0.0021	0.6380	0.5241			
1564	Intercept	-0.0041	0.0013	-3.0770	0.0024	0.056	$3.916_{(3, 198)}$	0.0096
	GTYP	0.0050	0.0015	3.2610	0.0013			
	SEX	0.0027	0.0019	1.4010	0.1628			
	G X S	-0.0034	0.0021	-1.5590	0.1205			

Table 3.4: F statistics for the overall model for the association between the C302000T SNP, crosses and sexes from 5000 permutations.

Cross	Sex	$F_{(df, df)}$	P-value	P-value - perm.*
1564	m	$1.288_{(1, 99)}$	0.2591	0.2610
	f	$9.36_{(1, 99)}$	0.0029	0.0020
5144	m	$2.524_{(1, 99)}$	0.1156	0.1106
	f	$6.226_{(1, 99)}$	0.0144	0.0128

*P-value- perm: P-value derived from 5000 permutations of the data (see methods).

Looking at the effects of the C30200T polymorphism on the C1 region on the samples we see that the average of the lines with a C at position 30200 is higher than those with T (Figure 3.4). The difference between the two genotypes is fairly constant across sexes and crosses. There may be slight effects of sex in cross 5144 (see table 3.4), but it is not formally significant (p=0.0939). These results are consistent with the effects of C30200T on C1 in other studies (Palsson *et al*. 2005) and we will elaborate more on that in the discussion.

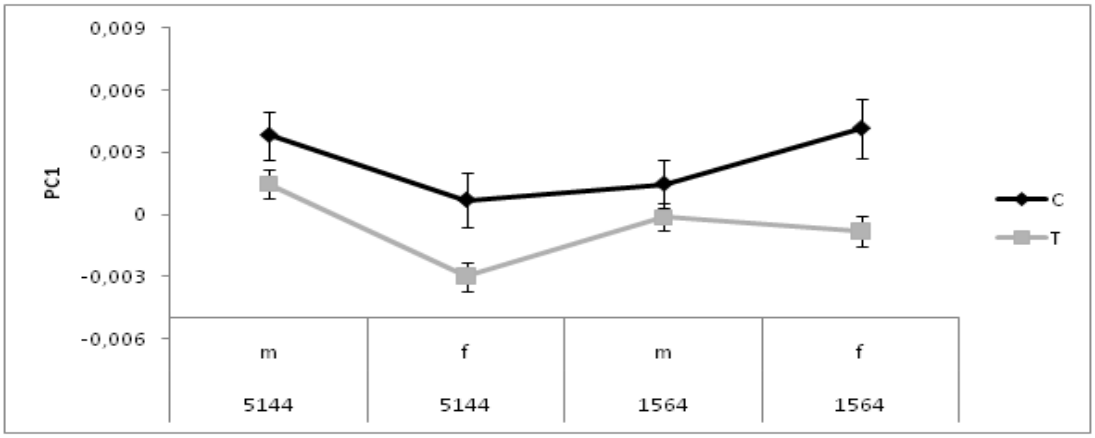

Figure 3.4: Effects of the T30200C polymorphism on C1 (PC1) in the samples. Each point represents the mean estimate plus or minus the standard error unit. (See appendix C table 1 for full table)

Crucially, we only observed this association if we worked on principal components extracted for the crosses separately. As can be inferred from above (See section - *Description of variation in traits*, figures 3.1 and 3.2) then the shape of the entire wing is very strongly affected by cross. If we focus on the C region then we see how different the first principal component of the C1 region if it is calculated for the whole population or crosses 5144 and 1564 separately (Figure 3.4). In the figure 3.5 we are observing two extremes of C1 in the whole populations and between crosses. For the whole population then, C1 responds both to the region and the placement of the crossveins. The C1 calculated for the crosses separately is almost entirely responding to the relative placement of crossveins (Figure 3.5 B and C), as it did in earlier studies (Palsson and Gibson 2004).

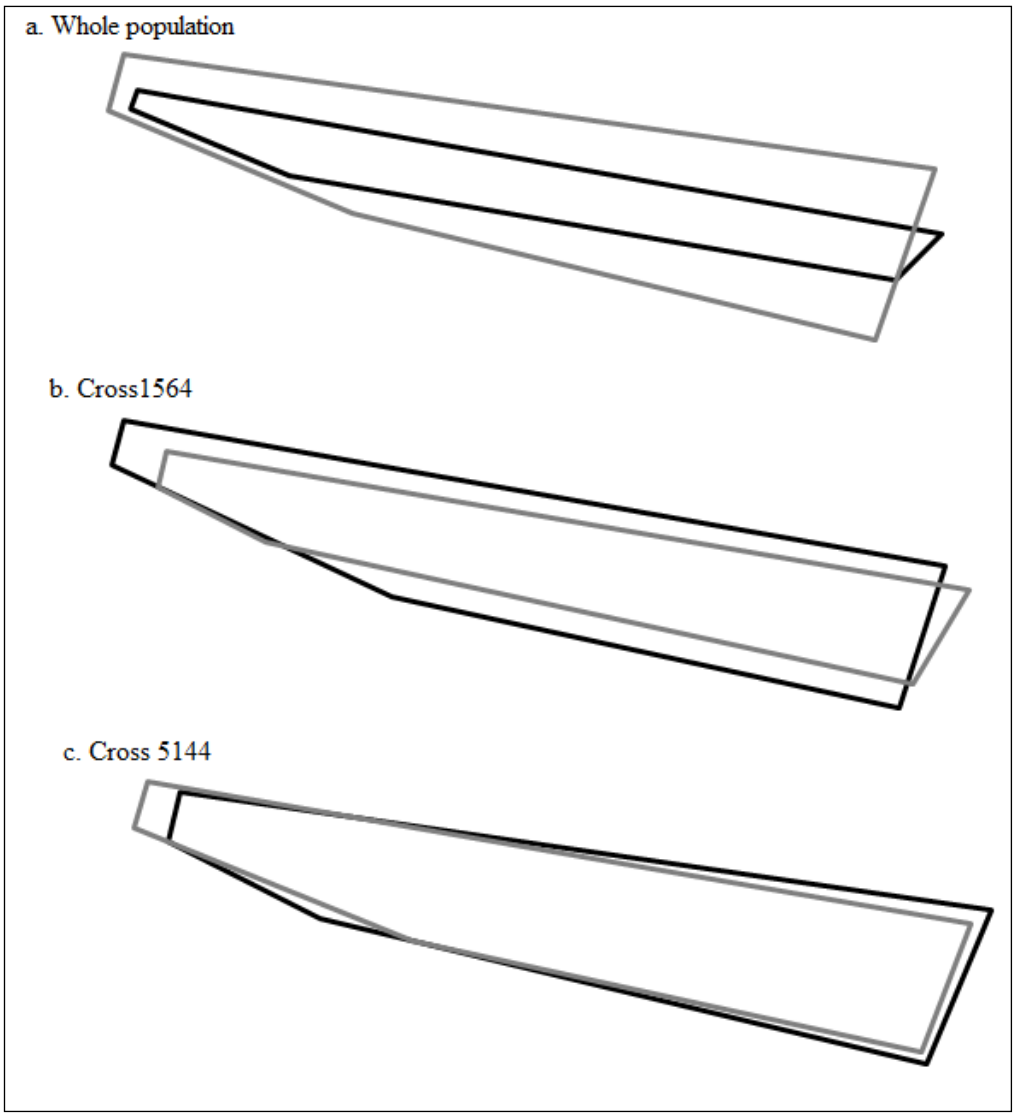

Figure 3.5: C1 region shape differences between two extremes for (a) all the samples (b) cross 1564 (c) cross 5144. Black lines represent the positive values and grey lines represent the negative values.

Test for association between *Egfr* polymorphism and extra veins

We wanted to determine if the *Egfr* polymorphism affected the formation of extra veins. This was evaluated with linear models, for each cross separately. The results showed no significant association between the 4 *Egfr* polymorphism SNPs and the extra vein A (evA) (see appendix D, table 1). With the extra vein B (evB), there was an impact of SNP 8535 and 8541. This association is between the SNP Sp08535 and Sp08541, and evB that depended on the cross (Table 3.5). In cross 5144 the genotype effect was significant (p=0.03) but nothing in the 1564 cross (presumably because the evB was not variable there). The interaction of genotype and sex was not formally significant (p=0.12), but the distribution of means and their standard errors suggest sex dependence of the effect. The two SNPs (Sp08535 and Sp08541) are in very high LD (Dworkin *et al*. 2003). No other significant association was found between the *Egfr* polymorphism

and evB (see appendix D, table 2). In figure 3.6 we see the effect of the SNP Sp08535-Sp08541 on evB in the samples.

Table 3.5: Linear model for the association between the SNP (Sp08535 and Sp08541) and the evB.

Cross	SNP	Term	Estimate	SD	t	p-value
c5144	Sp08535	Intercept	0.0394	0.0164	2.41	0.02
		GTYP	0.0449	0.0211	2.13	0.03
		SEX	0.0122	0.0232	0.53	0.60
		G X S	-0.0464	0.0299	-1.55	0.12
		R-squared:	0.0313			
		F(num,den):	1.906(3,177)	P:	1.30E-001	
c5144	Sp08541	Intercept	0.0844	0.0132	6.39	0.00
		GTYP	-0.0460	0.0208	-2.21	0.03
		SEX	-0.0342	0.0188	-1.82	0.07
		G X S	0.0461	0.0295	1.56	0.12
		R-squared:	0.03271			
		F(num,den):	2.018(3,179)	P:	1.13E-001	

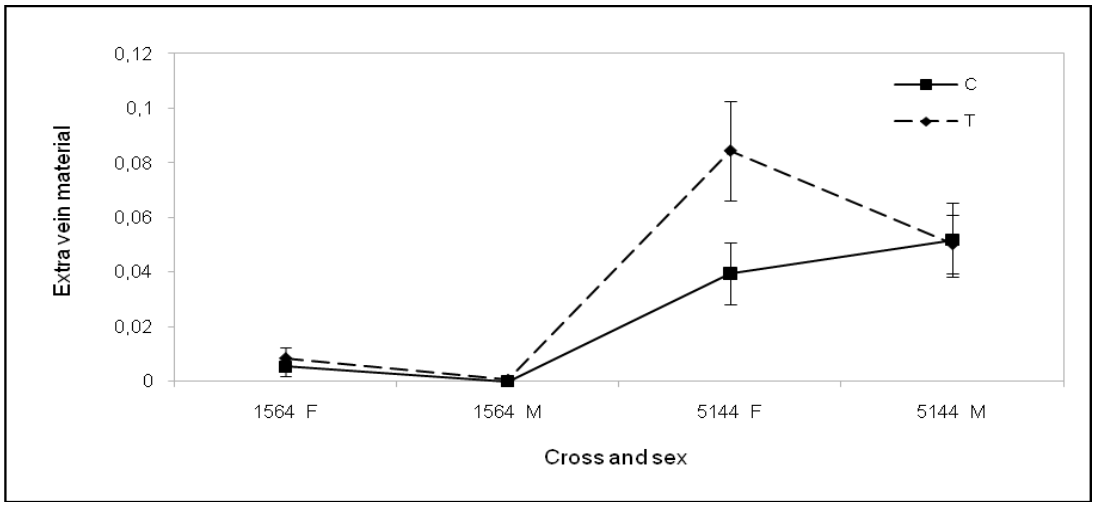

Figure 3.6: Effects of the Sp08535-08541 SNP on the extra vein B (evB) in the samples. Each point represents the mean estimate plus or minus the standard error unit.

Lastly, I studied the extra vein C1 (evC1). There is a significant association between the SNP Sp01335 (C30200T) and evC1. The polymorphism shows an association with evC1 in cross 1564 (p=0,03) but not with cross 5144 (p=0,36) (Table 3.6). The polymorphism also shows an association with evC1 when considering only the sexes (p=0,05 in the males and p=0,02 in the females) (Table 3.6). Figure 3.7 shows the effect of the Sp1335 SNP (C30200T) on the evC1 in the samples. We see that lines with T have (on average) more extravein material in this location than those with a C. This is seen both in the females and the males of cross 1564. Extra vein evC1 does not form in

cross 5144, thus we have no power to evaluate it with those data. Furthermore, none of the other SNPs affected evC1 (see appendix D, table 3).

Table 3.6: Linear model for the association between the SNP Sp01335 (C30200T) and the evC1.

Cross	SNP	Term	Estimate	SD	t	p-value
c1564	Sp01335	Intercept	0.0068	0.0029	2.32	0.02
		GTYP	0.0074	0.0033	2.22	0.03
		SEX	-0.0068	0.0041	-1.64	0.10
		G X S	-0.0049	0.0047	-1.04	0.30
		R-squared:	0.1528,			
		F(num,den):	11.54(3,192)	P:	5.44E-007	
c5144	Sp01335	Intercept	0.0005	0.0003	1.48	0.14
		GTYP	-0.0003	0.0004	-0.92	0.36
		SEX	-0.0005	0.0005	-1.04	0.30
		G X S	0.0006	0.0005	1.18	0.24
		R-squared:	0.007823,			
		F(num,den):	0.4705(3,179)	P:	7.03E-001	
Sex	**SNP**	**Term**	**Estimate**	**SD**	**t**	**p-value**
Males	Sp01335	Intercept	0.0000	0.0011	0.00	1.00
		GTYP	0.0025	0.0013	1.94	0.05
		Cross	0.0000	0.0016	0.00	1.00
		G X C	-0.0022	0.0019	-1.19	0.23
		R-squared:	0.04524,			
		F(num,den):	2.922(3,185)	P:	3.53E-002	
Females	Sp01335	Intercept	0.0068	0.0028	2.46	0.01
		GTYP	0.0074	0.0032	2.35	0.02
		Cross	-0.0063	0.0040	-1.60	0.11
		G X C	-0.0077	0.0045	-1.71	0.09
		R-squared:	0.2055,			
		F(num,den):	16.03(3,186)	P:	2.59E-009	

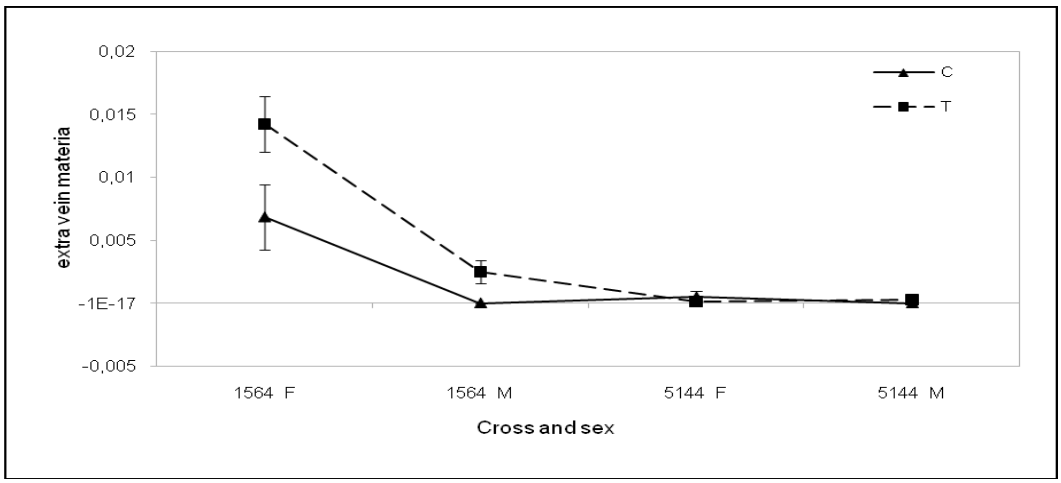

Figure 3.7: Effects of the Sp1335 SNP (C30200T) on the extra vein C1 (evC1) in the samples. Each point represents the mean estimate plus or minus the standard error unit.

Discussion

Description of variation in traits

From our results we see that there is a difference between the crosses in shape and size, and this is presumably due to the presence of the *narrow* allele in cross 1564 which causes elongated wings and this makes the wings of cross 1564 to be longer than the wings of cross 5144. Other possible causes may be other genetic differences between the two strains. This dimorphism means that the different regions (B, C and D) of the wings of cross 1564 are longer than those of cross 5144. The crosses also differ with the presence of the extra veins, with cross 1564 having more pronounced extra veins in their wings. The extra vein evA is very common among both crosses, however the length of the vein seems to depend on the cross and the sex as cross 5144 and the females from both crosses have longer veins. The quantity of the extra veins evB and evC1 depends on the cross and sex, such that evB is found mostly in the females of cross 5144 and evC1 in the females of cross 1564. The extra vein evC2 and evD are very rare and found mostly in the females of cross 1564, however, the amount of them found is not enough to evaluate if their presence is dependent on the cross or sex.

Comparing this with the correlation analysis it shows that the extra veins are independent of each other and do not in any way influence or affect the occurrence of each other. The genetic perturbance by E1 manifests in different ways such that the extra veins in the different part of the wing are not dependent although they are all enhanced by the same factor ($Egfr^{E1}$). That could suggest that correlation analyses on large phenotype matricies will not have a lot of power to find common agents influencing threshold traits.

Factors influencing the formation of the extra veins

The strongest factors influencing extra vein length are the size of the whole and parts of wing. What is interesting is that the extra veins show the strongest correlation to the size of the wing region in which they can be found, which shows that the location of the extra vein is also influenced by the size of the wing regions. The exceptions are for evC2 and evD and this is probably due to the fact that only very few were found (only one in 6325 individuals of the extra vein evD was found). Subdividing according to cross and sex resulted in a slightly different correlation matrix. Only the extra vein evA showed the strongest correlation to the size of the whole wing and the size of the parts of the wing. This is probably because a lot of the extra vein occurred in both the crosses and sexes (see table 3.1). The other extra veins showed no correlation to the size of the whole/parts of wing, and this is probably because subdividing the whole population according to the cross and sex reduces the number of extra vein in the groups.

Other factors also influenced the formation of extra veins. Cross, sex, and genotype affects the occurrence of extra veins and the amount of vein material in the extra veins evA, evB and evC1. Cross affects the presence or absence of an extra vein, however, this is only for the extra vein evB and evC1. For the extra vein evA, cross does not have any effect on its presence in the wings. Sex on the other hand seems to have the

strongest effect on the extra veins. Therefore, although cross allows for the presence of the extra vein, sex seems to determine both its presence and length. This is seen as the females of both crosses always have the most and longest extra vein for evA, evB and evC1

Association between the *Egfr* polymorphism and shape of region C

The association between the *Egfr* polymorphism and the C region (Palsson and Gibson 2004) is confirmed here. The results show a significant association between the C30200T SNP and the shape of the C region for both crosses 1564 and 5144. We determined that this was due to the C at SNP position 30200 has on average higher values than those with the T. This is also consistent with the studies of Palson *et al.* (2005) where they found that only the polymorphism T30200C showed a significant association and had a consistent effect on the wing shape. The shape component of the C1 region captured is the relative distance between the two crossveins. This is also consistent with earlier studies by Palsson and Gibson (2004) where a loss of function of Egfr signalling decreases the spacing between the anterior and posterior crossveins changing the shape of the C1 region on the wing. Here we are observing a gain of function of the Egfr signalling which resulted in increasing the spacing between the anterior and posterior crossvein (Figure 3.5) and thereby changing the shape if the C1 region on the wing.

Association between the *Egfr* polymorphism and the extra veins

After demonstrating that the C30200T **(**Sp01335**)** affects the shape of the C region, we next evaluated whether *Egfr* polymorphisms affected the formation of extra veins. We also evaluated other SNPs, Sp08196, Sp08535 and Sp08541 are known to contribute to the eye roughness phenotype in *Drosophila* (Dworkin *et al*. 2003). The SNPs Sp08535 are Sp08541 are in very high LD. There was no association between the SNPs and evA which is strange as E1 is supposed to induce the formation of extra veins in the A region. We would anticipate variants in *Egfr* to be able to modulate the extent of extra vein formation in this part of the wing. But these results suggest that the genetic variation extra vein formation, in the A region of the wing is not attributatble to these 4 mutations in *Egfr*. With the results obtained we can only presume that there might be other variants (in *Egfr* or in other interacting loci) affecting the formation of evA in the region, however this requires further study.

We found a tentative association between the SNPs Sp0853 and Sp08541, and evB in cross 5144. This is intriguing as these SNPs are known to affect eye roughness. However this association might be due to chance as we see the females of the cross having an unusual high C compared to the males. Due to this and the fact that sex and cross dependent associations replicate poorly (Palsson *et al.* 2005) we are treating this result with caution. It is fair to assume that test resulted in a false association between these SNPs and extra vein evB.

A priori we thought the highest chance of an association between Egfr variants and extra veins might be for SNP Sp01335 (C30200T) and evC1. Also as the trait was most pronounced in cross 1564 (almost absent from 5144 - see table 3.1), we expected the association to show up in the former cross. The results were consistent with these predictions (see table 3.6). The significant association obtained when considering only the sexes shows that the extra vein is dependent on both cross and sex and additionally confirms that cross and sex does have an effect on extra veins as was stated above.

On the whole, although different associations were obtained between the SNPs and the extraveins, there is an association between the *Egfr* polymorphism and the formation of extra veins. The same SNP affects the continuous trait and the manifestation of a threshold trait. This clearly implicates the role of *Egfr* signalling in both placement of veins, and also in the formation of the vein material.

Conclusion

In conclusion, the C30200T polymorphism affects both the shape of the wing and the formation of extra veins in *Drosophila*. The T allele of 30200 causes extra vein material to form on the wing, however this depends on the genetic background and the sex of the individual. As this polymorphism also as an effect on wing shape, there is a "genetic coupling" of the quantitative trait (wing shape) and the threshold character (extra vein material). This genetic coupling is exposed using a gain of function of the E1 allele of the *Egfr* gene.

In human genetics we could probably utilize this to understand the relationship between an underlying liability and threshold traits, an example will be understanding the relationship between cholesterol levels (underlying liability) and heart attack (threshold trait). Another example would be between insulin levels and diabetes. Understanding such relationships can help us understand the biology of these diseases which could assist in obtaining preventive measures/treatments against them. Also in plant and animal breeding, breeding lines to manipulate underlying variables underlying threshold points can probably bring out hidden favourable traits, however, this would involve determining which genes and traits are to be manipulated.

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Appendix

Appendix A

Histograms showing the distribution of the size of the total area, B, C and D area of the wing, and the length of the extra veins evA, evB,evC1, evC2 and evD across the whole population.

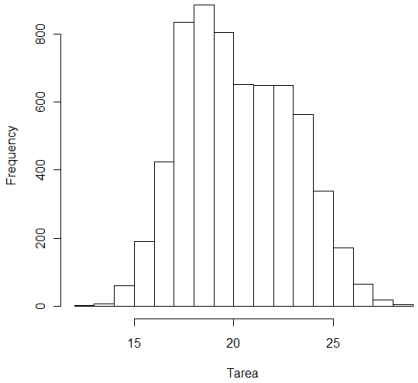

Figure 1: Size distribution of the whole wing across both cross 5144 and 1564

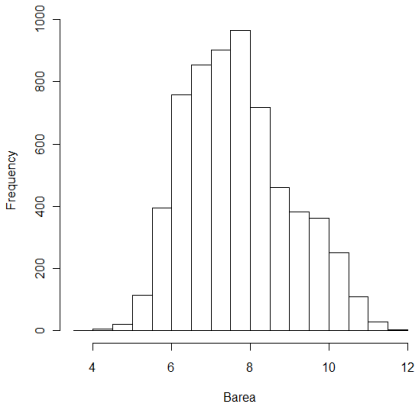

Figure 2: Size distribution of the B area across both cross 5144 and 1564

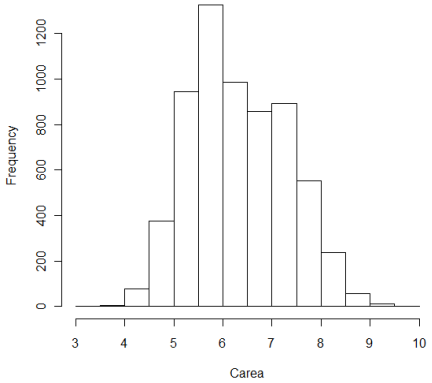

Figure 3: Size distribution of the C area across both cross 5144 and 1564

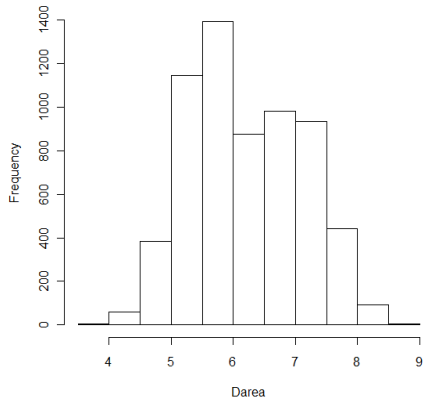

Figure 4: Size distribution of the D area across both cross 5144 and 1564

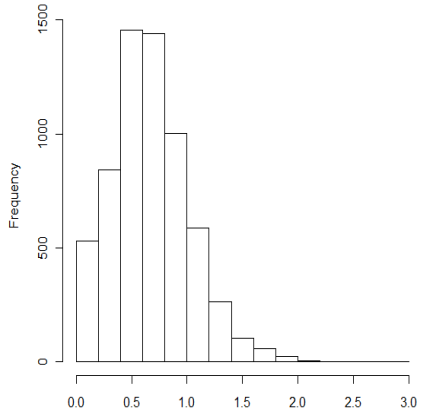

Figure 5: Length distribution of the extra vein evA across both cross 5144 and 1564

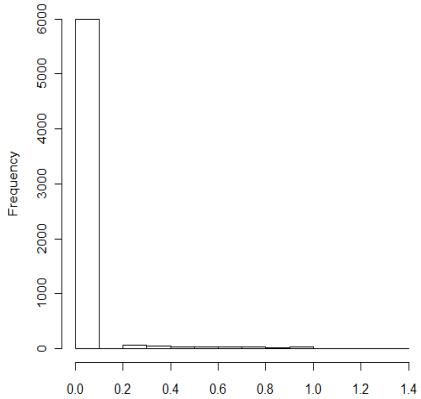

Figure 6: Length distribution of the extra vein evB across both cross 5144 and 1564

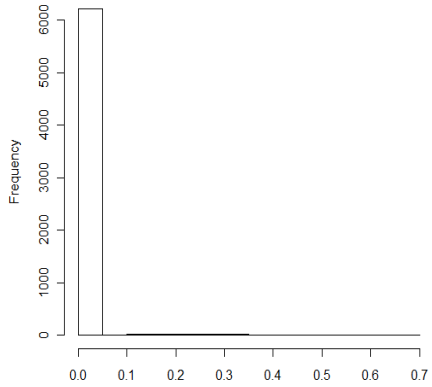

Figure 7: Length distribution of the extra vein evC1 across both cross 5144 and 1564

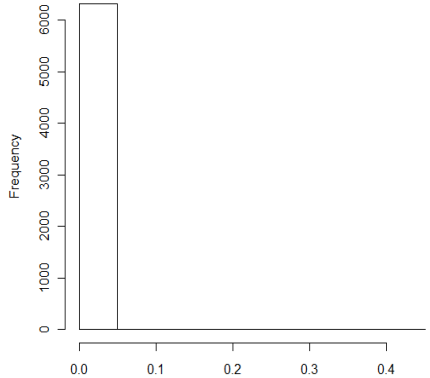

Figure 8: Length distribution of the extra vein evC2 across both cross 5144 and 1564

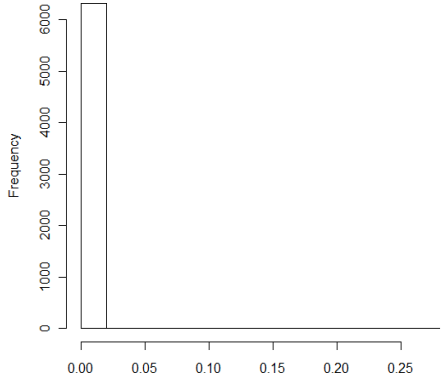

Figure 9: Length distribution of the extra vein evD across both cross 5144 and 1564

Appendix B

Tables showing the correlation matrix between the extra veins, areas and other variables. The boxes shaded gray signify a correlation above 0.1.

Table 1: Parametric (Pearsons) correlation matrix of all whole population from both crosses

	evA	evB	evC1	evC2	evD	Tarea	Barea	Carea	Darea	W1	W2	W3	W4	W5	W6	B1	B2	B3	C1	C2	C3	D1	D2	D3
evA	1.00	0.04	0.01	0.02	0.00	0.29	0.31	0.20	0.21	-0.16	0.01	0.06	0.14	0.00	-0.03	-0.16	-0.02	-0.13	-0.14	0.00	-0.05	0.13	0.14	0.01
evB	0.04	1.00	-0.01	0.00	0.00	0.10	0.13	0.07	0.03	-0.18	0.06	0.02	0.03	-0.05	0.06	-0.15	-0.07	-0.05	-0.18	-0.10	-0.04	0.16	0.07	-0.03
evC2	0.01	-0.01	1.00	0.00	0.03	0.04	-0.04	0.08	0.09	0.11	0.03	-0.03	0.06	-0.02	0.02	0.11	-0.02	-0.05	0.08	0.05	-0.05	-0.10	0.02	0.00
evC3	0.02	0.00	0.00	1.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	-0.03	-0.03	0.00	0.00	0.01	0.00	0.03	0.01	0.01	0.01	0.00	-0.03	-0.01
evD	0.00	0.00	0.03	0.00	1.00	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.01	0.02	0.02	-0.01	0.01	0.02
Tarea	0.29	0.10	0.04	0.00	0.01	1.00	0.82	0.87	0.88	-0.25	0.06	0.05	0.38	-0.16	0.06	-0.22	-0.05	-0.34	-0.25	0.01	-0.21	0.22	0.34	-0.07
Barea	0.31	0.13	-0.04	0.00	0.01	0.82	1.00	0.46	0.50	-0.63	-0.02	0.12	0.28	-0.04	0.00	-0.63	-0.02	-0.23	-0.54	-0.08	-0.09	0.58	0.33	-0.06
Carea	0.20	0.07	0.08	0.01	0.00	0.87	0.46	1.00	0.86	0.05	0.14	0.01	0.36	-0.19	0.12	0.10	-0.10	-0.33	-0.04	0.03	-0.23	-0.07	0.27	-0.08
Darea	0.21	0.03	0.09	0.00	0.01	0.88	0.50	0.86	1.00	0.12	0.07	-0.03	0.35	-0.23	0.06	0.15	-0.02	-0.33	0.09	0.13	-0.25	-0.09	0.24	-0.05
W1	-0.16	-0.18	0.11	0.01	0.01	-0.25	-0.63	0.05	0.12	1.00	0.00	0.00	0.00	0.00	0.00	0.97	0.10	0.02	0.88	0.24	-0.03	-0.95	-0.13	0.01
W2	0.01	0.06	0.03	0.00	0.00	0.06	-0.02	0.14	0.07	0.00	1.00	0.00	0.00	0.00	0.00	0.11	-0.99	-0.04	0.02	-0.10	-0.11	-0.13	0.15	-0.04
W3	0.06	0.02	-0.03	-0.03	0.00	0.05	0.12	0.01	-0.03	0.00	0.00	1.00	0.00	0.00	0.00	-0.16	-0.04	-0.06	0.16	-0.50	0.04	-0.17	0.57	0.04
W4	0.14	0.03	0.06	-0.03	0.00	0.38	0.28	0.36	0.35	0.00	0.00	0.00	1.00	0.00	0.00	0.03	0.07	-0.79	-0.16	0.38	-0.23	-0.05	0.61	-0.06
W5	0.00	-0.05	-0.02	0.00	0.02	-0.16	-0.04	-0.19	-0.23	0.00	0.00	0.00	0.00	1.00	0.00	-0.07	-0.01	0.40	-0.16	0.44	0.31	-0.10	0.02	0.12
W6	-0.03	0.06	0.02	0.00	0.00	0.06	0.00	0.12	0.06	0.00	0.00	0.00	0.00	0.00	1.00	0.08	0.03	0.37	-0.25	0.28	-0.16	0.03	0.49	0.03
B1	-0.16	-0.15	0.11	0.01	0.01	-0.22	-0.63	0.10	0.15	0.97	0.11	-0.16	0.03	-0.07	0.08	1.00	0.00	0.00	0.80	0.28	-0.05	-0.90	-0.15	-0.01
B2	-0.02	-0.07	-0.02	0.00	0.00	-0.05	-0.02	-0.10	-0.02	0.10	-0.99	-0.04	0.07	-0.01	0.03	0.00	1.00	0.00	0.03	0.16	0.01	0.03	-0.14	0.03
B3	-0.13	-0.05	-0.05	0.03	0.01	-0.34	-0.23	-0.33	-0.33	0.02	-0.04	-0.06	-0.79	0.40	0.37	0.00	0.00	1.00	0.03	0.09	0.23	0.01	-0.30	0.06
C1	-0.14	-0.18	0.08	0.01	0.01	-0.25	-0.54	-0.04	0.09	0.88	0.02	0.16	-0.16	-0.16	-0.25	0.80	0.03	0.03	1.00	0.00	0.00	-0.84	-0.20	-0.14
C2	0.00	-0.10	0.05	0.01	0.02	0.01	-0.08	0.03	0.13	0.24	-0.10	-0.50	0.38	0.44	0.28	0.28	0.16	0.09	0.00	1.00	0.00	-0.16	0.10	0.31
C3	-0.05	-0.04	-0.05	0.01	0.02	-0.21	-0.09	-0.23	-0.25	-0.03	-0.11	0.04	-0.23	0.31	-0.16	-0.05	0.01	0.23	0.00	0.00	1.00	0.09	-0.16	0.11
D1	0.13	0.16	-0.10	0.00	-0.01	0.22	0.58	-0.07	-0.09	-0.95	-0.13	-0.17	-0.05	-0.10	0.03	-0.90	0.03	0.01	-0.84	-0.16	0.09	1.00	0.00	0.00
D2	0.14	0.07	0.02	-0.03	0.01	0.34	0.33	0.27	0.24	-0.13	0.15	0.57	0.61	0.02	0.49	-0.15	-0.14	-0.30	-0.20	0.10	-0.16	0.00	1.00	0.00
D3	0.01	-0.03	0.00	-0.01	0.02	-0.07	-0.06	-0.08	-0.05	0.01	-0.04	0.04	-0.06	0.12	0.03	-0.01	0.03	0.06	-0.14	0.31	0.11	0.00	0.00	1.00

Table 2: Non-parametric (Spearmans) correlation matrix of all whole population from both crosses

	evA	evB	evC1	evC2	evD	Tarea	Barea	Carea	Darea	W1	W2	W3	W4	W5	W6	B1	B2	B3	C1	C2	C3	D1	D2	D3
evA	1.00	0.04	-0.01	0.02	0.00	0.29	0.32	0.20	0.20	-0.16	0.00	0.07	0.14	-0.02	-0.04	-0.17	-0.01	-0.13	-0.15	-0.02	-0.06	0.15	0.13	0.01
evB	0.04	1.00	-0.02	0.00	-0.01	0.09	0.14	0.07	0.00	-0.21	0.08	0.04	0.02	-0.05	0.06	-0.16	-0.10	-0.05	-0.22	-0.12	-0.04	0.19	0.08	-0.01
evC1	-0.01	-0.02	1.00	0.00	0.05	0.05	-0.04	0.09	0.09	0.11	0.03	-0.02	0.05	-0.02	0.02	0.12	-0.01	-0.04	0.08	0.06	-0.05	-0.11	0.00	0.01
evC2	0.02	0.00	0.00	1.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	-0.02	-0.02	0.00	0.00	0.01	0.00	0.02	0.01	0.01	0.01	0.00	-0.02	-0.02
evD	0.00	-0.01	0.05	0.00	1.00	0.01	0.01	0.00	0.01	0.01	-0.01	0.01	0.00	0.02	0.00	0.00	0.01	0.00	0.01	0.02	0.01	-0.01	0.00	0.02
Tarea	0.29	0.09	0.05	0.01	0.01	1.00	0.79	0.88	0.89	-0.18	0.07	0.04	0.39	-0.16	0.07	-0.14	-0.06	-0.36	-0.24	0.02	-0.22	0.21	0.34	-0.08
Barea	0.32	0.14	-0.04	0.00	0.01	0.79	1.00	0.46	0.47	-0.57	0.00	0.14	0.26	-0.05	0.01	-0.58	-0.05	-0.23	-0.56	-0.11	-0.09	0.58	0.35	-0.06
Carea	0.20	0.07	0.09	0.01	0.00	0.88	0.46	1.00	0.86	0.07	0.12	-0.01	0.37	-0.18	0.12	0.14	-0.09	-0.35	-0.05	0.04	-0.24	-0.06	0.28	-0.08
Darea	0.20	0.00	0.09	0.01	0.01	0.89	0.47	0.86	1.00	0.15	0.06	-0.05	0.37	-0.23	0.06	0.20	-0.01	-0.34	0.09	0.13	-0.26	-0.08	0.24	-0.05
W1	-0.16	-0.21	0.11	0.00	0.01	-0.18	-0.57	0.07	0.15	1.00	-0.07	0.01	0.04	0.00	-0.01	0.92	0.16	-0.01	0.87	0.21	-0.04	-0.92	-0.10	-0.02
W2	0.00	0.08	0.03	0.00	-0.01	0.07	0.00	0.12	0.06	-0.07	1.00	0.01	0.01	0.02	0.01	0.09	-0.99	-0.03	0.01	-0.10	-0.12	-0.11	0.15	-0.03
W3	0.07	0.04	-0.02	-0.02	0.01	0.04	0.14	-0.01	-0.05	0.01	0.01	1.00	-0.02	-0.03	0.00	-0.21	-0.06	-0.06	0.10	-0.52	0.03	-0.11	0.53	0.04
W4	0.14	0.02	0.05	-0.02	0.00	0.39	0.26	0.37	0.37	0.04	0.01	-0.02	1.00	-0.01	0.00	0.09	0.05	-0.79	-0.15	0.36	-0.22	-0.04	0.59	-0.07
W5	-0.02	-0.05	-0.02	0.00	0.02	-0.16	-0.05	-0.18	-0.23	0.00	0.02	-0.03	-0.01	1.00	-0.01	-0.09	-0.02	0.38	-0.14	0.41	0.32	-0.10	0.00	0.11
W6	-0.04	0.06	0.02	0.00	0.00	0.07	0.01	0.12	0.06	-0.01	0.01	0.00	0.00	-0.01	1.00	0.13	0.03	0.34	-0.24	0.26	-0.16	0.05	0.47	0.03
B1	-0.17	-0.16	0.12	0.01	0.00	-0.14	-0.58	0.14	0.20	0.92	0.09	-0.21	0.09	-0.09	0.13	1.00	0.02	-0.04	0.75	0.26	-0.08	-0.84	-0.11	-0.04
B2	-0.01	-0.10	-0.01	0.00	0.01	-0.06	-0.05	-0.09	-0.01	0.16	-0.99	-0.06	0.05	-0.02	0.03	0.02	1.00	0.00	0.05	0.16	0.02	0.02	-0.14	0.03
B3	-0.13	-0.05	-0.04	0.02	0.00	-0.36	-0.23	-0.35	-0.34	-0.01	-0.03	-0.06	-0.79	0.38	0.34	-0.04	0.00	1.00	0.04	0.07	0.24	0.00	-0.31	0.07
C1	-0.15	-0.22	0.08	0.01	0.01	-0.24	-0.56	-0.05	0.09	0.87	0.01	0.10	-0.15	-0.14	-0.24	0.75	0.05	0.04	1.00	0.02	-0.01	-0.83	-0.23	-0.13
C2	-0.02	-0.12	0.06	0.01	0.02	0.02	-0.11	0.04	0.13	0.21	-0.10	-0.52	0.36	0.41	0.26	0.26	0.16	0.07	0.02	1.00	0.01	-0.15	0.09	0.30
C3	-0.06	-0.04	-0.05	0.01	0.01	-0.22	-0.09	-0.24	-0.26	-0.04	-0.12	0.03	-0.22	0.32	-0.16	-0.08	0.02	0.24	-0.01	0.01	1.00	0.09	-0.16	0.10
D1	0.15	0.19	-0.11	0.00	-0.01	0.21	0.58	-0.06	-0.08	-0.92	-0.11	-0.11	-0.04	-0.10	0.05	-0.84	0.02	0.00	-0.83	-0.15	0.09	1.00	0.05	0.00
D2	0.13	0.08	0.00	-0.02	0.00	0.34	0.35	0.28	0.24	-0.10	0.15	0.53	0.59	0.00	0.47	-0.11	-0.14	-0.31	-0.23	0.09	-0.16	0.05	1.00	-0.01
D3	0.01	-0.01	0.01	-0.02	0.02	-0.08	-0.06	-0.08	-0.05	-0.02	-0.03	0.04	-0.07	0.11	0.03	-0.04	0.03	0.07	-0.13	0.30	0.10	0.00	-0.01	1.00

Table 3: Parametric (Pearsons) correlation matrix of the males of cross 5144

	evA	evB	evC1	evC2	evD	Tarea	Barea	Carea	Darea	W1	W2	W3	W4	W5	W6	B1	B2	B3	C1	C2	C3	D1	D2	D3
evA	1.00	0.01	0.02	NA	-0.03	0.18	0.20	0.10	0.16	-0.04	-0.12	0.07	0.09	0.00	-0.03	-0.14	0.12	-0.07	0.01	0.04	0.00	0.05	0.06	0.02
evB	0.01	1.00	0.06	NA	-0.01	0.02	-0.04	0.09	0.00	-0.10	0.13	0.01	0.01	-0.08	0.05	0.05	-0.12	-0.06	-0.12	-0.09	-0.03	0.02	0.04	0.03
evC1	0.02	0.06	1.00	NA	0.00	-0.01	-0.01	0.00	-0.03	-0.12	0.06	-0.10	0.03	0.06	0.03	-0.02	-0.06	0.01	-0.11	0.04	0.01	0.08	-0.01	-0.02
evC	NA	NA	NA	1.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
evD	-0.03	-0.01	0.00	NA	1.00	0.02	0.05	-0.03	0.02	-0.01	0.02	0.01	-0.02	0.05	0.02	-0.04	-0.01	0.07	0.02	0.05	-0.01	0.00	0.02	0.02
Tarea	0.18	0.02	-0.01	NA	0.02	1.00	0.88	0.84	0.91	-0.03	-0.06	-0.02	0.05	-0.08	0.15	0.07	0.07	-0.01	-0.12	-0.01	-0.08	0.07	0.10	-0.05
Barea	0.20	-0.04	-0.01	NA	0.05	0.88	1.00	0.54	0.73	-0.09	-0.08	0.02	0.06	0.17	-0.01	-0.20	0.07	0.08	0.04	0.14	0.09	0.03	0.06	-0.05
Carea	0.10	0.09	0.00	NA	-0.03	0.84	0.54	1.00	0.70	-0.05	0.01	-0.07	0.07	-0.20	0.28	0.29	0.01	-0.10	-0.39	-0.20	-0.15	-0.05	0.11	-0.07
Darea	0.16	0.00	-0.03	NA	0.02	0.91	0.73	0.70	1.00	0.09	-0.09	-0.02	0.00	-0.27	0.16	0.16	0.11	-0.03	0.04	-0.01	-0.20	0.24	0.08	-0.01
W1	-0.04	-0.10	-0.12	NA	-0.01	-0.03	-0.09	-0.05	0.09	1.00	-0.54	0.05	0.01	0.00	-0.03	0.62	0.54	0.01	0.39	0.07	0.01	-0.46	-0.10	-0.04
W2	-0.12	0.13	0.06	NA	0.02	-0.06	-0.08	0.01	-0.09	-0.54	1.00	0.09	-0.28	0.01	0.01	-0.16	-0.99	0.20	-0.04	-0.19	-0.04	-0.07	0.07	0.04
W3	0.07	0.01	-0.10	NA	0.01	-0.02	0.02	-0.07	-0.02	0.05	0.09	1.00	-0.15	-0.31	-0.09	-0.33	-0.12	-0.11	0.38	-0.50	0.03	-0.35	0.36	0.14
W4	0.09	0.01	0.03	NA	-0.02	0.05	0.06	0.07	0.00	0.01	-0.28	-0.15	1.00	0.15	-0.01	0.06	0.32	-0.70	-0.35	0.47	-0.01	0.03	0.51	0.02
W5	0.00	-0.08	0.06	NA	0.05	-0.08	0.17	-0.20	-0.27	0.00	0.01	-0.31	0.15	1.00	-0.13	-0.12	-0.02	0.27	-0.27	0.54	0.40	-0.16	-0.12	0.06
W6	-0.03	0.05	0.03	NA	0.02	0.15	-0.01	0.28	0.16	-0.03	0.01	-0.09	-0.01	-0.13	1.00	0.41	0.03	0.45	-0.49	0.29	-0.22	0.19	0.63	-0.03
B1	-0.14	0.05	-0.02	NA	-0.04	0.07	-0.20	0.29	0.16	0.62	-0.16	-0.33	0.06	-0.12	0.41	1.00	0.20	0.11	-0.18	0.07	0.00	-0.11	0.06	-0.13
B2	0.12	-0.12	-0.06	NA	-0.01	0.07	0.07	0.01	0.11	0.54	-0.99	-0.12	0.32	-0.02	0.03	0.20	1.00	-0.20	0.00	0.21	-0.03	0.07	-0.05	-0.04
B3	-0.07	-0.06	0.01	NA	0.07	-0.01	0.08	-0.10	-0.03	0.01	0.20	-0.11	-0.70	0.27	0.45	0.11	-0.20	1.00	0.04	0.14	0.06	0.00	-0.10	-0.07
C1	0.01	-0.12	-0.11	NA	0.02	-0.12	0.04	-0.39	0.04	0.39	-0.04	0.38	-0.35	-0.27	-0.49	-0.18	0.00	0.04	1.00	-0.32	-0.01	-0.26	-0.28	-0.25
C2	0.04	-0.09	0.04	NA	0.05	-0.01	0.14	-0.20	-0.01	0.07	-0.19	-0.50	0.47	0.54	0.29	0.07	0.21	0.14	-0.32	1.00	0.04	0.16	0.32	0.31
C3	0.00	-0.03	0.01	NA	-0.01	-0.08	0.09	-0.15	-0.20	0.01	-0.04	0.03	-0.01	0.40	-0.22	0.00	-0.03	0.06	-0.01	0.04	1.00	0.12	-0.11	0.03
D1	0.05	0.02	0.08	NA	0.00	0.07	0.03	-0.05	0.24	-0.46	-0.07	-0.35	0.03	-0.16	0.19	-0.11	0.07	0.00	-0.26	0.16	0.12	1.00	0.01	-0.02
D2	0.06	0.04	-0.01	NA	0.02	0.10	0.06	0.11	0.08	-0.10	0.07	0.36	0.51	-0.12	0.63	0.06	-0.05	-0.10	-0.28	0.32	-0.11	0.01	1.00	0.08
D3	0.02	0.03	-0.02	NA	0.02	-0.05	-0.05	-0.07	-0.01	-0.04	0.04	0.14	0.02	0.06	-0.03	-0.13	-0.04	-0.07	-0.25	0.31	0.03	-0.02	0.08	1.00

Table 4: Parametric (Pearsons) correlation matrix of the females of cross 5144

	evA	evB	evC1	evC2	evD	Tarea	Barea	Carea	Darea	W1	W2	W3	W4	W5	W6	B1	B2	B3	C1	C2	C3	D1	D2	D3
evA	1.00	0.00	-0.02	NA	NA	0.17	0.17	0.11	0.18	0.00	-0.12	0.08	0.11	-0.03	-0.07	-0.09	0.11	-0.14	0.03	0.00	0.03	0.05	0.06	0.02
evB	0.00	1.00	0.03	NA	NA	0.01	-0.05	0.09	0.01	-0.09	0.11	-0.02	-0.03	-0.08	0.10	0.09	-0.10	0.00	-0.12	-0.11	-0.06	0.07	0.03	-0.06
evC1	-0.02	0.03	1.00	NA	NA	0.01	0.01	-0.01	0.03	0.07	-0.09	0.02	0.00	-0.01	-0.05	-0.01	0.09	-0.02	0.06	-0.02	-0.02	-0.01	-0.05	-0.03
evC2	NA	NA	NA	1.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
evD	NA	NA	NA	NA	1.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tarea	0.17	0.01	0.01	NA	NA	1.00	0.89	0.85	0.92	0.07	-0.09	0.09	0.10	-0.07	0.09	0.09	0.09	-0.10	-0.06	-0.03	-0.03	-0.06	0.14	0.00
Barea	0.17	-0.05	0.01	NA	NA	0.89	1.00	0.57	0.77	0.01	-0.11	0.12	0.10	0.15	-0.05	-0.17	0.10	0.01	0.08	0.12	0.13	-0.05	0.11	0.01
Carea	0.11	0.09	-0.01	NA	NA	0.85	0.57	1.00	0.72	0.03	0.01	0.04	0.12	-0.17	0.24	0.29	0.02	-0.16	-0.34	-0.20	-0.13	-0.16	0.17	-0.03
Darea	0.18	0.01	0.03	NA	NA	0.92	0.77	0.72	1.00	0.19	-0.13	0.09	0.06	-0.24	0.07	0.17	0.14	-0.12	0.09	-0.03	-0.11	0.09	0.11	0.04
W1	0.00	-0.09	0.07	NA	NA	0.07	0.01	0.03	0.19	1.00	-0.57	0.07	0.00	0.01	-0.04	0.62	0.57	0.01	0.41	0.07	0.03	-0.51	-0.14	0.00
W2	-0.12	0.11	-0.09	NA	NA	-0.09	-0.11	0.01	-0.13	-0.57	1.00	0.04	-0.27	0.05	0.07	-0.12	-0.99	0.20	-0.13	-0.17	0.00	0.01	0.10	0.02
W3	0.08	-0.02	0.02	NA	NA	0.09	0.12	0.04	0.09	0.07	0.04	1.00	-0.16	-0.33	-0.06	-0.30	-0.08	-0.10	0.38	-0.54	0.06	-0.31	0.36	0.12
W4	0.11	-0.03	0.00	NA	NA	0.10	0.10	0.12	0.06	0.00	-0.27	-0.16	1.00	0.12	-0.09	0.03	0.30	-0.73	-0.30	0.45	-0.03	0.04	0.50	-0.03
W5	-0.03	-0.08	-0.01	NA	NA	-0.07	0.15	-0.17	-0.24	0.01	0.05	-0.33	0.12	1.00	-0.11	-0.08	-0.05	0.29	-0.30	0.56	0.41	-0.13	-0.12	0.08
W6	-0.07	0.10	-0.05	NA	NA	0.09	-0.05	0.24	0.07	-0.04	0.07	-0.06	-0.09	-0.11	1.00	0.42	-0.04	0.47	-0.49	0.23	-0.17	0.16	0.60	0.00
B1	-0.09	0.09	-0.01	NA	NA	0.09	-0.17	0.29	0.17	0.62	-0.12	-0.30	0.03	-0.08	0.42	1.00	0.16	0.13	-0.16	0.08	-0.01	-0.19	0.05	-0.07
B2	0.11	-0.10	0.09	NA	NA	0.09	0.10	0.02	0.14	0.57	-0.99	-0.08	0.30	-0.05	-0.04	0.16	1.00	-0.21	0.09	0.19	-0.08	-0.02	-0.08	-0.03
B3	-0.14	0.00	-0.02	NA	NA	-0.10	0.01	-0.16	-0.12	0.01	0.20	-0.10	-0.73	0.29	0.47	0.13	-0.21	1.00	0.01	0.12	0.09	0.00	-0.14	-0.01
C1	0.03	-0.12	0.06	NA	NA	-0.06	0.08	-0.34	0.09	0.41	-0.13	0.38	-0.30	-0.30	-0.49	-0.16	0.09	0.01	1.00	-0.32	0.02	-0.24	-0.29	-0.26
C2	0.00	-0.11	-0.02	NA	NA	-0.03	0.12	-0.20	-0.03	0.07	-0.17	-0.54	0.45	0.56	0.23	0.08	0.19	0.12	-0.32	1.00	0.10	0.16	0.27	0.31
C3	0.03	-0.06	-0.02	NA	NA	-0.03	0.13	-0.13	-0.11	0.03	0.00	0.06	-0.03	0.41	-0.17	-0.01	-0.08	0.09	0.02	0.10	1.00	0.15	-0.03	0.07
D1	0.05	0.07	-0.01	NA	NA	-0.06	-0.05	-0.16	0.09	-0.51	0.01	-0.31	0.04	-0.13	0.16	-0.19	-0.02	0.00	-0.24	0.16	0.15	1.00	0.05	-0.02
D2	0.06	0.03	-0.05	NA	NA	0.14	0.11	0.17	0.11	-0.14	0.10	0.36	0.50	-0.12	0.60	0.05	-0.08	-0.14	-0.29	0.27	-0.03	0.05	1.00	0.04
D3	0.02	-0.06	-0.03	NA	NA	0.00	0.01	-0.03	0.04	0.00	0.02	0.12	-0.03	0.08	0.00	-0.07	-0.03	-0.01	-0.26	0.31	0.07	-0.02	0.04	1.00

Table 5: Parametric (Pearsons) correlation matrix of the males of cross 1564

	evA	evB	evC1	evC2	evD	Tarea	Barea	Carea	Darea	W1	W2	W3	W4	W5	W6	B1	B2	B3	C1	C2	C3	D1	D2	D3
evA	1.00	0.02	0.00	NA	-0.03	0.26	0.28	0.17	0.17	-0.11	0.14	0.05	0.01	0.04	0.00	-0.11	-0.15	-0.01	-0.06	-0.03	-0.02	0.01	0.07	0.03
evB	0.02	1.00	0.00	NA	0.00	0.06	0.06	0.06	0.02	-0.03	0.04	0.00	0.04	0.00	-0.02	-0.02	-0.04	-0.04	-0.03	0.00	-0.02	0.01	0.02	0.01
evC1	0.00	0.00	1.00	NA	0.00	0.02	0.00	0.02	0.02	0.00	0.02	0.00	0.03	-0.03	-0.01	0.00	-0.01	-0.03	0.01	0.00	-0.04	0.00	0.02	0.00
evC2	NA	NA	NA	1.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
evD	-0.03	0.00	0.00	NA	1.00	0.04	0.05	0.02	0.04	0.00	0.00	0.01	0.00	-0.01	-0.03	-0.01	0.00	-0.02	0.01	-0.03	0.00	0.00	-0.01	-0.01
Tarea	0.26	0.06	0.02	NA	0.04	1.00	0.75	0.78	0.87	-0.04	0.07	0.06	0.05	-0.11	0.06	0.00	-0.06	-0.10	-0.06	-0.12	-0.09	0.02	0.08	0.00
Barea	0.28	0.06	0.00	NA	0.05	0.75	1.00	0.26	0.51	-0.10	0.03	0.09	-0.01	-0.04	0.02	-0.12	-0.03	-0.02	-0.03	-0.09	-0.02	0.04	0.07	0.01
Carea	0.17	0.06	0.02	NA	0.02	0.78	0.26	1.00	0.64	-0.01	0.10	0.03	0.07	-0.08	0.06	0.07	-0.08	-0.10	-0.07	-0.10	-0.09	-0.03	0.08	-0.01
Darea	0.17	0.02	0.02	NA	0.04	0.87	0.51	0.64	1.00	0.01	0.04	0.02	0.05	-0.16	0.05	0.07	-0.02	-0.12	-0.03	-0.09	-0.13	0.02	0.05	0.00
W1	-0.11	-0.03	0.00	NA	0.00	-0.04	-0.10	-0.01	0.01	1.00	-0.07	0.51	0.28	0.00	-0.07	0.77	0.10	-0.27	0.67	-0.18	-0.04	-0.85	0.38	-0.18
W2	0.14	0.04	0.02	NA	0.00	0.07	0.03	0.10	0.04	-0.07	1.00	-0.02	0.11	0.08	-0.03	0.22	-0.99	-0.10	-0.05	-0.04	-0.14	-0.19	0.19	-0.05
W3	0.05	0.00	0.00	NA	0.01	0.06	0.09	0.03	0.02	0.51	-0.02	1.00	0.06	0.01	-0.02	0.02	-0.01	-0.10	0.59	-0.51	0.05	-0.67	0.65	-0.03
W4	0.01	0.04	0.03	NA	0.00	0.05	-0.01	0.07	0.05	0.28	0.11	0.06	1.00	0.03	0.03	0.36	-0.03	-0.78	-0.12	0.33	-0.22	-0.34	0.60	-0.07
W5	0.04	0.00	-0.03	NA	-0.01	-0.11	-0.04	-0.08	-0.16	0.00	0.08	0.01	0.03	1.00	0.05	-0.19	-0.08	0.42	-0.25	0.51	0.25	-0.20	0.08	0.11
W6	0.00	-0.02	-0.01	NA	-0.03	0.06	0.02	0.06	0.05	-0.07	-0.03	-0.02	0.03	0.05	1.00	0.14	0.06	0.36	-0.46	0.34	-0.14	0.09	0.46	0.09
B1	-0.11	-0.02	0.00	NA	-0.01	0.00	-0.12	0.07	0.07	0.77	0.22	0.02	0.36	-0.19	0.14	1.00	-0.16	-0.34	0.27	-0.02	-0.11	-0.55	0.25	-0.18
B2	-0.15	-0.04	-0.01	NA	0.00	-0.06	-0.03	-0.08	-0.02	0.10	-0.99	-0.01	-0.03	-0.08	0.06	-0.16	1.00	0.05	0.01	0.08	0.03	0.16	-0.17	0.04
B3	-0.01	-0.04	-0.03	NA	-0.02	-0.10	-0.02	-0.10	-0.12	-0.27	-0.10	-0.10	-0.78	0.42	0.36	-0.34	0.05	1.00	-0.13	0.16	0.22	0.26	-0.29	0.09
C1	-0.06	-0.03	0.01	NA	0.01	-0.06	-0.03	-0.07	-0.03	0.67	-0.05	0.59	-0.12	-0.25	-0.46	0.27	0.01	-0.13	1.00	-0.52	0.06	-0.55	0.10	-0.36
C2	-0.03	0.00	0.00	NA	-0.03	-0.12	-0.09	-0.10	-0.09	-0.18	-0.04	-0.51	0.33	0.51	0.34	-0.02	0.08	0.16	-0.52	1.00	0.05	0.23	0.07	0.38
C3	-0.02	-0.02	-0.04	NA	0.00	-0.09	-0.02	-0.09	-0.13	-0.04	-0.14	0.05	-0.22	0.25	-0.14	-0.11	0.03	0.22	0.06	0.05	1.00	0.13	-0.12	0.17
D1	0.01	0.01	0.00	NA	0.00	0.02	0.04	-0.03	0.02	-0.85	-0.19	-0.67	-0.34	-0.20	0.09	-0.55	0.16	0.26	-0.55	0.23	0.13	1.00	-0.55	0.17
D2	0.07	0.02	0.02	NA	-0.01	0.08	0.07	0.08	0.05	0.38	0.19	0.65	0.60	0.08	0.46	0.25	-0.17	-0.29	0.10	0.07	-0.12	-0.55	1.00	-0.01
D3	0.03	0.01	0.00	NA	-0.01	0.00	0.01	-0.01	0.00	-0.18	-0.05	-0.03	-0.07	0.11	0.09	-0.18	0.04	0.09	-0.36	0.38	0.17	0.17	-0.01	1.00

Table 6: Parametric (Pearsons) correlation matrix of the females of cross 1564

	evA	evB	evC1	evC2	evD	Tarea	Barea	Carea	Darea	W1	W2	W3	W4	W5	W6	B1	B2	B3	C1	C2	C3	D1	D2	D3
evA	1.00	0.03	0.01	0.03	0.04	0.18	0.26	0.05	0.05	-0.09	0.02	0.01	0.05	0.07	-0.02	-0.11	-0.01	-0.02	-0.09	0.05	-0.03	0.01	0.03	0.06
evB	0.03	1.00	-0.02	0.00	0.00	0.01	0.02	0.03	-0.04	-0.05	0.00	0.00	0.01	0.01	0.02	-0.05	-0.01	0.00	-0.04	0.01	-0.01	0.02	0.02	0.00
evC1	0.01	-0.02	1.00	-0.01	0.03	0.01	-0.02	0.02	0.03	0.02	0.01	-0.03	0.06	-0.01	0.04	0.05	0.00	-0.04	-0.03	0.04	-0.04	-0.02	0.03	0.01
evC2	0.03	0.00	-0.01	1.00	0.00	0.00	0.00	0.01	-0.02	-0.02	-0.01	-0.04	-0.06	0.01	0.00	-0.01	0.00	0.07	0.00	0.01	0.03	0.04	-0.05	-0.03
evD	0.04	0.00	0.03	0.00	1.00	0.01	0.01	0.00	0.01	0.02	-0.02	0.00	0.02	0.02	0.01	0.02	0.02	-0.01	-0.02	0.03	0.04	-0.01	0.01	0.04
Tarea	0.18	0.01	0.01	0.00	0.01	1.00	0.64	0.74	0.82	-0.07	-0.03	0.06	0.03	-0.05	0.11	-0.06	0.03	-0.02	-0.09	-0.05	-0.06	0.04	0.09	0.04
Barea	0.26	0.02	-0.02	0.00	0.01	0.64	1.00	0.04	0.25	-0.16	-0.05	0.07	0.00	0.08	0.04	-0.22	0.05	0.05	-0.12	0.02	-0.06	0.07	0.07	0.08
Carea	0.05	0.03	0.02	0.01	0.00	0.74	0.04	1.00	0.63	0.05	0.05	0.06	0.05	-0.10	0.11	0.10	-0.04	-0.07	-0.02	-0.10	-0.04	-0.05	0.10	-0.01
Darea	0.05	-0.04	0.03	-0.02	0.01	0.82	0.25	0.63	1.00	-0.02	-0.05	0.00	0.02	-0.13	0.09	0.02	0.06	-0.05	-0.04	-0.04	-0.03	0.07	0.03	0.02
W1	-0.09	-0.05	0.02	-0.02	0.02	-0.07	-0.16	0.05	-0.02	1.00	0.02	0.54	0.18	-0.03	0.04	0.77	0.01	-0.18	0.69	-0.31	-0.02	-0.85	0.40	-0.15
W2	0.02	0.00	0.01	-0.01	-0.02	-0.03	-0.05	0.05	-0.05	0.02	1.00	-0.05	0.11	-0.07	-0.05	0.34	-0.99	-0.17	0.04	-0.11	-0.14	-0.21	0.15	-0.10
W3	0.01	0.00	-0.03	-0.04	0.00	0.06	0.07	0.06	0.00	0.54	-0.05	1.00	0.08	0.17	0.08	0.02	0.03	-0.03	0.53	-0.47	0.01	-0.71	0.71	0.05
W4	0.05	0.01	0.06	-0.06	0.02	0.03	0.00	0.05	0.02	0.18	0.11	0.08	1.00	0.06	0.03	0.22	-0.04	-0.75	-0.18	0.33	-0.22	-0.28	0.59	-0.02
W5	0.07	0.01	-0.01	0.01	0.02	-0.05	0.08	-0.10	-0.13	-0.03	-0.07	0.17	0.06	1.00	0.12	-0.32	0.07	0.45	-0.30	0.49	0.19	-0.22	0.21	0.17
W6	-0.02	0.02	0.04	0.00	0.01	0.11	0.04	0.11	0.09	0.04	-0.05	0.08	0.03	0.12	1.00	0.15	0.08	0.38	-0.36	0.32	-0.13	0.00	0.49	0.07
B1	-0.11	-0.05	0.05	-0.01	0.02	-0.06	-0.22	0.10	0.02	0.77	0.34	0.02	0.22	-0.32	0.15	1.00	-0.29	-0.30	0.37	-0.18	-0.05	-0.51	0.17	-0.22
B2	-0.01	-0.01	0.00	0.00	0.02	0.03	0.05	-0.04	0.06	0.01	-0.99	0.03	-0.04	0.07	0.08	-0.29	1.00	0.13	-0.07	0.14	0.03	0.18	-0.13	0.09
B3	-0.02	0.00	-0.04	0.07	-0.01	-0.02	0.05	-0.07	-0.05	-0.18	-0.17	-0.03	-0.75	0.45	0.38	-0.30	0.13	1.00	-0.11	0.18	0.21	0.18	-0.22	0.07
C1	-0.09	-0.04	-0.03	0.00	-0.02	-0.09	-0.12	-0.02	-0.04	0.69	0.04	0.53	-0.18	-0.30	-0.36	0.37	-0.07	-0.11	1.00	-0.59	0.07	-0.55	0.10	-0.35
C2	0.05	0.01	0.04	0.01	0.03	-0.05	0.02	-0.10	-0.04	-0.31	-0.11	-0.47	0.33	0.49	0.32	-0.18	0.14	0.18	-0.59	1.00	0.03	0.31	0.05	0.35
C3	-0.03	-0.01	-0.04	0.03	0.04	-0.06	-0.06	-0.04	-0.03	-0.02	-0.14	0.01	-0.22	0.19	-0.13	-0.05	0.03	0.21	0.07	0.03	1.00	0.13	-0.13	0.08
D1	0.01	0.02	-0.02	0.04	-0.01	0.04	0.07	-0.05	0.07	-0.85	-0.21	-0.71	-0.28	-0.22	0.00	-0.51	0.18	0.18	-0.55	0.31	0.13	1.00	-0.58	0.11
D2	0.03	0.02	0.03	-0.05	0.01	0.09	0.07	0.10	0.03	0.40	0.15	0.71	0.59	0.21	0.49	0.17	-0.13	-0.22	0.10	0.05	-0.13	-0.58	1.00	0.05
D3	0.06	0.00	0.01	-0.03	0.04	0.04	0.08	-0.01	0.02	-0.15	-0.10	0.05	-0.02	0.17	0.07	-0.22	0.09	0.07	-0.35	0.35	0.08	0.11	0.05	1.00

Appendix C

Table 1: Table showing the mean number with the standard errors and variance of the C and T genotypes of T30200C SNP among the crosses and sexes

		C		T		Variance		number of lines	
Cross	**Sex**	**mean**	**SE**	**mean**	**SE**	**C**	**T**	**C**	**T**
5144	m	0.0038124	0.0011549	0.0014629	0.0007019	0.0000267	0.0000365	20	74
5144	f	0.0006982	0.0012993	-0.0029850	0.0006945	0.0000355	0.0000357	21	74
1564	m	0.0014729	0.0011572	-0.0001309	0.0006719	0.0000295	0.0000357	22	79
1564	f	0.0041359	0.0014498	-0.0008196	0.0007535	0.0000462	0.0000448	22	79

Appendix D

Linear models for the association between four EGFR polymorphisms and the extraveins evA, evB and evC1.

Table 1: Linear model for the association between the *EGFR* polymorphism and the evA.

Cross	SNP	Term	Estimate	SD	t	p-value
5144	**Sp01335**	Intercept	0.7929	0.0423	18.77	0.00
		GTYP	0.0066	0.0481	0.14	0.89
		SEX	-0.1344	0.0605	-2.22	0.03
		G X S	-0.0099	0.0687	-0.14	0.89
		R-squared:	0.121			
		F(num,den):	8.21(3,179)	P:	3.78E-005	
1564	**Sp01335**	Intercept	0.6746	0.0548	12.31	0.00
		GTYP	0.0211	0.0622	0.34	0.74
		SEX	-0.0896	0.0775	-1.16	0.25
		G X S	-0.0601	0.0880	-0.68	0.50
		R-squared:	0.06906			
		F(num,den):	4.748(3,192)	P:	3.23E-003	
5144	**Sp08196**	Intercept	0.8296	0.0285	29.09	0.00
		GTYP	-0.0579	0.0408	-1.42	0.16
		SEX	-0.1730	0.0406	-4.26	0.00
		G X S	0.0618	0.0579	1.07	0.29
		R-squared:	0.1337			
		F(num,den):	8.793(3,171)	P:	1.87E-005	
1564	**Sp08196**	Intercept	0.7031	0.0371	18.95	0.00
		GTYP	-0.0244	0.0522	-0.47	0.64
		SEX	-0.1553	0.0525	-2.96	0.00
		G X S	0.0206	0.0738	0.28	0.78
		R-squared:	0.08085			
		F(num,den):	5.219(3,178)	P:	1.78E-003	
5144	**Sp08535**	Intercept	0.8222	0.0324	25.41	0.00
		GTYP	-0.0410	0.0416	-0.98	0.33
		SEX	-0.1642	0.0458	-3.59	0.00
		G X S	0.0370	0.0590	0.63	0.53
		R-squared:	0.1243			
		F(num,den):	8.372(3,177)	P:	3.10E-005	
1564	**Sp08535**	Intercept	0.7061	0.0415	17.01	0.00
		GTYP	-0.0350	0.0536	-0.65	0.51
		SEX	-0.1653	0.0587	-2.82	0.01
		G X S	0.0621	0.0758	0.82	0.41
		R-squared:	0.06327			
		F(num,den):	4.188(3,186)	P:	6.76E-003	
5144	**Sp08541**	Intercept	0.7813	0.0260	30.01	0.00
		GTYP	0.0414	0.0411	1.01	0.31
		SEX	-0.1272	0.0370	-3.44	0.00
		G X S	-0.0369	0.0582	-0.63	0.53
		R-squared:	0.1259			
		F(num,den):	8.592(3,179)	P:	2.33E-005	
1564	**Sp08541**	Intercept	0.6711	0.0337	19.89	0.00
		GTYP	0.0325	0.0529	0.62	0.54
		SEX	-0.1032	0.0477	-2.16	0.03
		G X S	-0.0597	0.0748	-0.80	0.43
		R-squared:	0.06306			
		F(num,den):	4.218(3,188)	P:	6.49E-003	
1564	**Sp08697**	Intercept	0.6977	0.0327	21.34	0.00
		GTYP	-0.0292	0.0547	-0.53	0.59
		SEX	-0.1412	0.0462	-3.05	0.00
		G X S	0.0200	0.0773	0.26	0.80
		R-squared:	0.06716			
		F(num,den):	4.463(3,186)	P:	4.71E-003	
5144	**Sp08697**	Intercept	0.7984	0.0259	30.84	0.00
		GTYP	-0.0043	0.0421	-0.10	0.92
		SEX	-0.1405	0.0366	-3.84	0.00
		G X S	-0.0090	0.0598	-0.15	0.88
		R-squared:	0.124			
		F(num,den):	8.261(3,175)	P:	3.59E-005	

Table 2: Linear model for the association between the *EGFR* polymorphism and the evB.

Cross:	SNP	Term	Estimate	SD	t	p-value:
c1564	**Sp01335**	Intercept	0.0082	0.0048	1.70	0.09
		GTYP	0.0009	0.0055	0.16	0.87
		SEX	-0.0082	0.0068	-1.20	0.23
		G X S	-0.0005	0.0077	-0.07	0.95
		R-squared:	0.03578			
		F(num,den):	2.375(3,192)	P:	7.15E-002	
c5144	**Sp01335**	Intercept	0.0852	0.0216	3.95	0.00
		GTYP	-0.0251	0.0245	-1.02	0.31
		SEX	-0.0472	0.0309	-1.53	0.13
		G X S	0.0408	0.0350	1.16	0.25
		R-squared:	0.01424			
		F(num,den):	0.8619(3,179)	P:	4.62E-001	
c1564	**Sp08196**	Intercept	0.0102	0.0033	3.12	0.00
		GTYP	-0.0041	0.0046	-0.90	0.37
		SEX	-0.0096	0.0046	-2.06	0.04
		G X S	0.0035	0.0065	0.53	0.60
		R-squared:	0.03543			
		F(num,den):	2.179(3,178)	P:	9.21E-002	
c5144	**Sp08196**	Intercept	0.0560	0.0138	4.07	0.00
		GTYP	0.0070	0.0197	0.36	0.72
		SEX	-0.0092	0.0196	-0.47	0.64
		G X S	-0.0006	0.0279	-0.02	0.98
		R-squared:	0.004012			
		F(num,den):	0.2296(3,171)	P:	8.76E-001	
c1564	**Sp08535**	Intercept	0.0053	0.0030	1.75	0.08
		GTYP	0.0032	0.0039	0.81	0.42
		SEX	-0.0053	0.0043	-1.24	0.22
		G X S	-0.0026	0.0055	-0.48	0.63
		R-squared:	0.03698			
		F(num,den):	2.381(3,186)	P:	7.10E-002	
c5144	**Sp08535**	Intercept	0.0394	0.0164	2.41	0.02
		GTYP	0.0449	0.0211	2.13	0.03
		SEX	0.0122	0.0232	0.53	0.60
		G X S	-0.0464	0.0299	-1.55	0.12
		R-squared:	0.0313			
		F(num,den):	1.906(3,177)	P:	1.30E-001	
c1564	**Sp08541**	Intercept	0.0084	0.0030	2.80	0.01
		GTYP	0.0016	0.0047	0.33	0.74
		SEX	-0.0079	0.0043	-1.85	0.07
		G X S	-0.0021	0.0067	-0.32	0.75
		R-squared:	0.03703			
		F(num,den):	2.41(3,188)	P:	6.84E-002	
c5144	**Sp08541**	Intercept	0.0844	0.0132	6.39	0.00
		GTYP	-0.0460	0.0208	-2.21	0.03
		SEX	-0.0342	0.0188	-1.82	0.07
		G X S	0.0461	0.0295	1.56	0.12
		R-squared:	0.03271			
		F(num,den):	2.018(3,179)	P:	1.13E-001	
c1564	**Sp08697**	Intercept	0.0082	0.0029	2.80	0.01
		GTYP	0.0021	0.0049	0.43	0.67
		SEX	-0.0082	0.0041	-1.98	0.05
		G X S	-0.0012	0.0069	-0.18	0.86
		R-squared:	0.03626			
		F(num,den):	2.333(3,186)	P:	7.56E-002	
c5144	**Sp08697**	Intercept	0.0546	0.0123	4.45	0.00
		GTYP	0.0165	0.0200	0.83	0.41
		SEX	-0.0057	0.0174	-0.33	0.74
		G X S	-0.0136	0.0284	-0.48	0.63
		R-squared:	0.007566			
		F(num,den):	0.4447(3,175)	P:	7.21E-001	

Table 3: Linear model for the association between the *EGFR* polymorphism and the evC1.

Cross	SNP:	Term	Estimate	SD	t	p-value:
c1564	**Sp01335**	Intercept	0.0068	0.0029	2.32	0.02
		GTYP	0.0074	0.0033	2.22	0.03
		SEX	-0.0068	0.0041	-1.64	0.10
		G X S	-0.0049	0.0047	-1.04	0.30
		R-squared:	0.1528,			
		F(num,den):	11.54(3,192)	P:	5.44E-007	
c5144	**Sp01335**	Intercept	0.0005	0.0003	1.48	0.14
		GTYP	-0.0003	0.0004	-0.92	0.36
		SEX	-0.0005	0.0005	-1.04	0.30
		G X S	0.0006	0.0005	1.18	0.24
		R-squared:	0.007823,			
		F(num,den):	0.4705(3,179)	P:	7.03E-001	
Sex	**SNP**	**Term**	**Estimate**	**SD**	**t**	**p-value:**
Males	**Sp01335**	Intercept	0.0000	0.0011	0.00	1.00
		GTYP	0.0025	0.0013	1.94	0.05
		Cross	0.0000	0.0016	0.00	1.00
		G X C	-0.0022	0.0019	-1.19	0.23
		R-squared:	0.04524,			
		F(num,den):	2.922(3,185)	P:	3.53E-002	
Females	**Sp01335**	Intercept	0.0068	0.0028	2.46	0.01
		GTYP	0.0074	0.0032	2.35	0.02
		Cross	-0.0063	0.0040	-1.60	0.11
		G X C	-0.0077	0.0045	-1.71	0.09
		R-squared:	0.2055,			
		F(num,den):	16.03(3,186)	P:	2.59E-009	
Cross	**SNP**	**Term**	**Estimate**	**SD**	**t**	**p-value:**
c1564	**Sp08196**	Intercept	0.0136	0.0021	6.53	0.00
		GTYP	-0.0029	0.0029	-1.00	0.32
		SEX	-0.0107	0.0029	-3.64	0.00
		G X S	0.0011	0.0041	0.27	0.79
		R-squared:	0.1248,			
		F(num,den):	8.459(3,178)	P:	2.76E-005	
c5144	**Sp08196**	Intercept	0.0002	0.0002	0.99	0.33
		GTYP	0.0000	0.0003	0.03	0.97
		SEX	0.0000	0.0003	0.02	0.99
		G X S	0.0000	0.0005	-0.01	0.99
		R-squared:	8.328e-06,			
		F(num,den):	0.0004747(3,171)	P:	1.00E+000	
c1564	**Sp08535**	Intercept	0.0124	0.0023	5.44	0.00
		GTYP	0.0001	0.0029	0.03	0.98
		SEX	-0.0111	0.0032	-3.44	0.00
		G X S	0.0007	0.0042	0.17	0.87
		R-squared:	0.1285,			
		F(num,den):	9.138(3,186)	P:	1.13E-005	
c5144	**Sp08535**	Intercept	0.0000	0.0002	0.00	1.00
		GTYP	0.0004	0.0003	1.15	0.25
		SEX	0.0003	0.0003	0.80	0.43
		G X S	-0.0005	0.0004	-1.01	0.31
		R-squared:	0.007829,			
		F(num,den):	0.4656(3,177)	P:	7.07E-001	
c1564	**Sp08541**	Intercept	0.0125	0.0019	6.71	0.00
		GTYP	0.0001	0.0029	0.04	0.97
		SEX	-0.0104	0.0026	-3.94	0.00
		G X S	-0.0004	0.0041	-0.10	0.92
		R-squared:	0.1258,			
		F(num,den):	9.022(3,188)	P:	1.30E-005	
c5144	**Sp08541**	Intercept	0.0004	0.0002	1.83	0.07
		GTYP	-0.0004	0.0003	-1.16	0.25
		SEX	-0.0002	0.0003	-0.63	0.53
		G X S	0.0004	0.0004	1.01	0.31
		R-squared:	0.007883,			
		F(num,den):	0.4741(3,179)	P:	7.01E-001	
c1564	**Sp08697**	Intercept	0.0123	0.0018	6.83	0.00
		GTYP	0.0009	0.0030	0.31	0.75
		SEX	-0.0100	0.0025	-3.93	0.00
		G X S	-0.0018	0.0043	-0.42	0.68
		R-squared:	0.1282,			
		F(num,den):	9.121(3,186)	P:	1.16E-005	
c5144	**Sp08697**	Intercept	0.0002	0.0002	0.90	0.37
		GTYP	0.0001	0.0003	0.36	0.72
		SEX	0.0000	0.0003	0.00	1.00
		G X S	0.0000	0.0005	0.02	0.99
		R-squared:	0.001545,			
		F(num,den):	0.09029(3,175)	P:	9.65E-001	