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Genesis versus epigenesis: the odd jobs of the *Polycomb* group of genes

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ABSTRACT Cells need the products of the *Polycomb* Group of genes (PcG) to keep, through development, the memory of their genetic determination. The pleiotropic mutant phenotypes of PcG genes strikingly resemble morphological traits, considered as taxonomic characters with phylogenetic meaning, used in Dipteran classification. I argue that spatial modulation of the multimeric complexes formed by PcG products has played a role or escorted the genesis of species.

KEY WORDS: *Polycomb group genes, gene regulation, PcG evolutive role, cladistics*

The *Polycomb* group *(PcG)* genes

The *PcG* genes were originally identified in *Drosophila* by their mutant phenotypes. At first glance all of them mimic multiple gains of function of homeotic *(HOM)* genes. Upon closer examination, not only the specificity of homeotic transformations and their frequency do not coincide, but some of them present mutant phenotypes that can hardly be attributed to alterations of the *HOM* gene expression. The most obvious common denominator and origin of many names is the transformation of the second leg into the first, most easily observed by the appearance of the prominent sex comb in the basitarsus of male mid-legs. However, by choosing other common phenotypes, one can define sub-groups including or excluding different combinatorial of *PcG* elements, or make groups with other elements not considered to belong to the *PcG*. If we choose the molecular approach to group them, as some interact directly to form molecular complexes (Franke *et al.,* 1992; Rastelli *et al.,* 1993) again we can imagine different combinations regulating different targets. Also, when antibodies have been produced against some of them, they stain polytenic chromosomes in largely coincident sites, corresponding to *HOM* or to other genes known to be silenced by *PcG* genes, but they do not have exactly the same pattern (Franke *et al.,* 1992; Messmer *et al.,* 1992; Rastelli *et al.,* 1993; Lonie *et al.,* 1994).

A lot of results have been collected from the study of the *PcG* genes and excellent reviews exist about their role, with comprehensive genetic, developmental and molecular results (Moehrle and Paro, 1994; Orlando and Paro, 1995; Paro, 1995; Simon, 1995; Pirrotta, 1997; Santamaría and Randsholt, 1998). Table 1 is a non-exhaustive list summarizing the most known.

Many *PcG* genes have been conserved during evolution. For instance, *Psc* has a murine homolog called *bmi-1* (Brunk *et al.,* 1991; Van Lohuizen *et al.,* 1991; Van der Lugt *et al.,* 1994,1996) which provokes, when mutated, pleiotropic posteriorly directed homeotic transformations that parallel the *PcG* mutant phenotypes in *Drosophila* (Alkema *et al.,* 1995). Mutants for this gene are related to tumorigenesis (Haupt *et al.,* 1993; Cohen *et al.,* 1996). Another homolog of *Psc* is *mel-18* (Kanno *et al.,* 1995; Akasama *et al.,* 1996), also considered as a tumor suppressor. *Polycomb* has a mouse homolog: M-33 (Pearce *et al.,* 1992) that alters cellular proliferation and patterning (Coré *et al.,* 1997) and can correct the lack of *Pc* product in transgenic flies (Müller *et al.,* 1995). In *Xenopus* the *Pc* and *Psc* homologous proteins form multimeric complexes, as in *Drosophila* (Reijnen *et al.,* 1995). The mouse homolog of *ph: rae-28* (Nomura *et al.,* 1994) is activated by treatment with retinoic acid. Other human proteins presenting homology with *ph* are HPH1 and HPH2, that also interact with *Bmi-1* (Gunster *et al.,* 1997). The product of *E(z)/pco* shares a domain, the SET, with the human mixed-lineage leukemia Mll/HRX/All-1 (Lawrence and Largman, 1992; Jones and Gelbart, 1993; Yu *et al.,* 1995; Hobert *et al.,* 1996a,b) and mammalian homologs of *E(z)* mediate gene silencing in transgenic flies (Laible *et al.,* 1997). On the other hand, the proteins encoded by *Pc, Psc* and *Su(z)2* genes can all mediate the repression of transcription in mammalian cells (Bunker and Kingston, 1994), again indicating that the structure and function of *PcG* genes have been conserved to a large extent during evolution. For review, see Schumacher and Magnuson (1997). Gene silencing is also necessary in plants (Jürgens, 1997). The localized expression of *AGAMOUS,* a homeotic gene of *Arabidopsis,* needs the expression of the gene *CURLY LEAF (ClF))* to prevent its ectopic expression (Goodrich *et al.,* 1997), *ClF* product has similarities with *E(z).*

Abbreviations used in this paper: PcG, Polycomb Group.

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As the combination of activation and repression of selector genes that gives rise to specific parts of an organism responds to transient signals, any multicellular organism undergoing development needs a mechanism to maintain the process of cellular determination through many rounds of cell division. Once established, such mechanism could well have been conserved during evolution; *HOM* genes have been conserved (Manak and Scott, 1994; Burke *et al.,* 1995) and the full set of *Hox* genes appears to have existed in the common ancestor of tetrapods and fishes (Duboule and Morata, 1994). If the primitive *Hox* expression patterns are useful to define units, their modulation would have contributed to the evolution of axial structures into a common plan (Carroll, 1995), the "zootype" of Slack *et al.* (1993) reviewed by Duboule (1994). Many targets of *PcG* genes have been conserved apart from H*ox* genes. The not yet described "rules" (not laws, not dogma) of "evolution tinkering with development" (Jacob, 1977,1993) would suggest that "cassettes" of regulatory cascades of gene activity could have been conserved (Jan and Jan, 1993). At least, a part of the *PcG* genes could have been kept on top of some of these cascades. As the group of words in a phrase that form a functional unit, these cascades can be called "syntagmata" (García Bellido, 1982). For Alberch (1980) "Unfortunately, recent evolutionary theory has been plagued by a strong reductionist approach (by opposition to holistic) which has led us to neglect the importance of the constraints imposed by higher order interaction at epigenetic and functional levels". For my part, I will in fact, as a counterpoint, indulge through this paper in the role of some epigenetic factors on evolution, mostly in a "reductionist" approach.

Epigenesis

The *Polycomb* Group of genes has been said to control the epigenetic transcriptional regulation by the establishment of a state of the chromatin that keeps homeotic and other selector genes silenced through cellular division (Moehrle and Paro, 1994; Pirrotta, 1995,1997). It must however be recognized that the use of the term epigenetic regulation suffers from the "age and usage" of the word "epigenesis". First used by Caspar Friedrich Wolf in 1759, in association with the word "theory" (by comparison to the theory of preformation), this word only means that "building an embryo had to be... a virtual force... not essentially different from a creative principle" (quoted from Balinsky, 1965). For Waddington (1966), the "epigenetic landscape" is a metaphor to describe a surface in which vector fields and slopes condition the possible fates of a cell in a morphogenetic territory. García Bellido and Capdevilla (1978) writing more specifically on the topic of initiation and maintenance of gene activity in developmental pathways, consider the epigenetic determinants as the external agents applied during development that may affect the identity of a group of cells with similar fate. They refer specifically to the phenocopying agents and in fact, curiously, the phenocopies induced at the blastoderm stage in the *Drosophila* embryo by a heat shock mimic the phenotypes of the PcG mutants (Santamaría, 1979). Recently, Hollyday (1994) has written an exhaustive review on epigenesis, and TIG dedicated a special issue (1997, 13, N° 8). For most authors the epigenetic systems are responsible for regulating the expression of particular specificities, they determine which of them will be selected from the genetic library and when they will be expressed. Inherently, they concern functions that cells may have the possibility to express but which are not indispensable for cellular life. All types of mechanisms conceivable to fulfil the commitment and inheritance of a given pattern of gene expression are compatible with this definition once the difference with developmental determination is awkwardly grasped. These facts led Løvtropt (1974) to write that "It appears unavoidable that everything, without exception, which happens in the embryo after fertilization, must be classified as epigenetic event". For a developmental geneticist, the meaning is faded.

With regard to the PcG genes, the fact of the matter is that the etymology (epi: upon, besides outer) makes unbalanced the sentence: "epigenetic transcriptional regulation" because it emphasizes the perspective from the PcG targets instead of the point of view from the PcG themselves and, in some way, suggests PcG factors not to be genetic. Eventually, most people would agree that the products of the *PcG* genes help to keep the memory of determination throughout development. They maintain a stable state of silencing upon many genes including homeotics, by making complexes that probably affect the chromatin. It was the lack of evidence for DNA binding by the PcG products and the assumed changes of chromatin that prompted the usage of the "epigenetic" adjective. Once initiated, this state is generally self-maintained through cell division. How this state pervades development is still a challenge.

TABLE 1

KNOWN MEMBERS OF THE *POLYCOMB* GROUP GENES

polyhomeotic (ph)	*Additional sex combs (Asx)*
cramped (crm)	*Polycomblike (Pcl)*
multi sex comb (mxc)	*Enhancer of zeste/polycombeotic (E(z)/pco)*
extra sex combs (esc)	*Polycomb (Pc)*
super sex combs (sxc)	*Sex comb on midleg (Scm)*
Posterior sex combs (Psc)	*Sex comb extra (Sce)*
Suppressor of zeste 2 (Su(z)2)	*pleiohomeotic/l(4)102EFc (pho)*

Genesis

Some important evolutionary events are likely to be changes in *Hox* usage that lead to the acquisition of new functions (Ahlberg, 1992; Slack *et al.,* 1993; Holland *et al.,* 1994; Averof and Akam, 1995; Sordino *et al.,* 1995; Tabin, 1995; Gibson and Hogness, 1996; Tautz, 1996; Sharkey *et al.,* 1997). A step forward would be that differences in the combinations formed by regulatory proteins caused new spatial or temporal pattern of expression of their targets. *Hox-C* genes in *Caenorhabditis* can be misled in their expression by altered local environments (Schnabel and Schnabel, 1997). Furthermore as *Hox* genes are expressed in temporal colinearity, heterochronies of gene activation may shift expression domains and induce changes in morphogenesis with an evolutive impact (Duboule, 1994). The fact that *Hox-d, 10, 11, 12* and *13* suffer a coordinate variation of expression simultaneously to form, as a novelty, the vertebrate digits (Duboule and Sordino, 1996) suggests the possibility that not necessarily a *cis* but a *trans* regulatory element could have played a role in this alteration, and by consequence, in the origin of specific morphogenetic process. If not necessarily causal in speciation, it could have been a concomitant event.

A constitutive gain of function of a selector gene in an individual can be expected to have frequently dramatic consequences. In comparison, changing the deployment of integrated cascades of gene regulation (cassette or syntagma), may be less disruptive and

could be at the origin of a new character or function by ectopic expression. The idea that the *PcG* genes may have played a role in evolution is based on the observation that a few mutant phenotypes of *PcG* genes coincide with specie's characteristics with taxonomic or systematic value on Dipterans.

I will now summarize a convergent bundle of results gained from the study of *PcG* genes and go thoroughly through two examples.

Cladistic characters of some PcG mutants

The method of "Phylogenetic systematics" used to classify species by Willi Hennig (1966) called -by Mayr- "cladism" tries to reflect the genealogy of organisms by considering only their phylogenetic relationship. The characters are considered by their genealogical meaning. "Apomorphic" -new, modified- characters can arise from "plesiomorphic" - ancestral - characters. The same "apomorphy" in two taxons, called "synapomorphy" suggests their monophyletic relationship. Slack *et al.* (1993) consider the zootype a "synapomorphy of the Animalia kingdom". Likewise, the same "plesiomorphy" in two taxons (five digits in *Homo* and *Elephas* for instance) is a "Symplesiomorphy" and suggests primitive links. A reminiscent idea ("spatial differentnesses in development, due to invisible physical and chemical conditions preceding the formation of a visible pattern", atavic or not) was called "prepattern" by Curt Stern (1954). The observations below follow, in my opinion, the same rationale. A cladistic classification of *Drosophilidae* has been given by Grimaldi (1990).

A frequently used version to subdivide the *Diptera* and place *Drosophila* (McAlpine 1989) is offered in Figure 1.

A plesiomorphic character seen in most *Muscomorpha* is the presence of three spermatecae (McAlpine, 1989). This character is present in mutants of *Pcl* and *pho* of *Drosophila melanogaster* (Duncan, 1982; Girton and Jeon, 1994). The explanation of this prepattern by the alteration of segmental identity due to effects of Pc mutants on *HOM* genes has been suggested by Duncan (1982).

Some *Scaptodrosophila* in particular *Scaptodrosophila victoria, Scaptodrosophila coracina* and *Scaptodrosophila pattersoni (*García Bellido, 1983) show the pattern of the second abdominal segment also in the first segment. Mutants of *ph,* possibly because of a gain of function -*Uab*- of a *HOM* gene, (Dura *et al.,* 1985) show the same pattern.

In the order of Dipterans, there is a general trend towards the shortening of the abdomen (McAlpine, 1989). The seventh tergite of males is present, a plesiomorphy, still visible in Scapto*Drosophila* or in some Sophophora as *Drosophila takahashii.* The *melanogaster* subgroup (Fig. 3) does not have a seventh tergite (Santamaría and García Bellido, 1972), but a small slender plate without bristles is still visible in *Drosophila erecta* and *orena*. Also, some mutations of loss of function of *Abd-B* cause this seventh tergite to reappear. The phylogenetic trend is exaggerated in most PcG mutants (Dura *et al.,* 1985) where the sixth abdominal segment tends to disappear because of its transformation towards the seventh, probably due to a gain of function of the *HOM* genes.

Order: *Diptera*
- Suborder: *Nematocera*
- Suborder: *Brachycera*
  - Infraorder: *Muscomorpha (= Cyclorrhapha)*
    - Section: *Aschiza*
    - Section: *Schizophora*
      - Subsection: *Calyptratae*
      - Subsection: *Acalyptratae*
        - Superfamily: *Ephydroidea*
          - Family: *Drosophilidae*
            - Subfamily: *Drosophilinae*
              - Genus: *Scaptodrosophila*
              - Genus: *Drosophila*
                - Subgenus: *Drosophila*
                - Subgenus: *Sophophora*

Fig. 1. The *Drosophila* lineage.

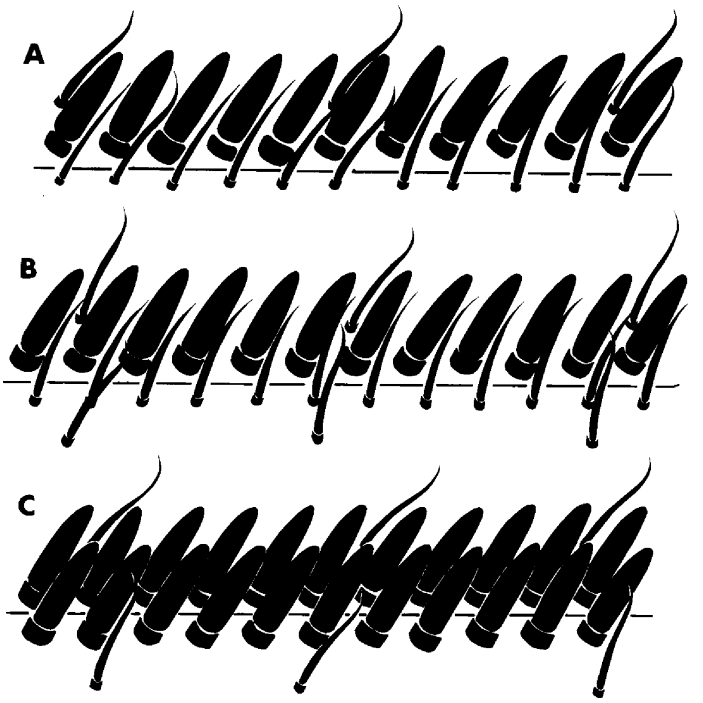


Fig. 2. Bristle pattern of the anterior margin of the wing. (A) *in Drosophila melanogaster,* **(B)** *in Drosophila affinis and* **(C)** *in some mutants (Dlw and PcG) of Drosophila melanogaster and in Drosophila gibberosa.*

Mutants of many *PcG* genes, for instance *ph* and *Asx,* lose the postpronotal bristles (Humerus). This is a plesiomorphy which differentiates more primitive *Muscomorpha*, the *Aschiza*, where they are absent, from most evolved, the *Schizophora* where they are present (McAlpine, 1989).

The wing margin

The *melanogaster* group has a pattern of bristles on the anterior margin of the wing formed by three rows of bristles (Fig. 2A), two of dorsal origin and one of ventral lineage (García Bellido and Merriam, 1971). For the two dorsal rows, the one called medial row is the most marginal and formed by "stout" short, thick, single inervated bristles (Hartenstein and Posakony, 1989). More interiorly is the dorsal row of slender, bent, chemosensory, multiple inervated bristles. Ventrally, a single row is formed by two types of bristles; straight single inervated and bent multiple inervated bristles. An analogy between the identical multiinervated bristles of both surfaces is suggested by the fact that some mutants of *scute [(Df(1)sc19)]* take out this type of bristles on both surfaces. The character "Triple row" is apomorphic from the "Four row" plesiomorphic character shown in Figure 2B. This suggestion is supported by different facts. One is that wild-

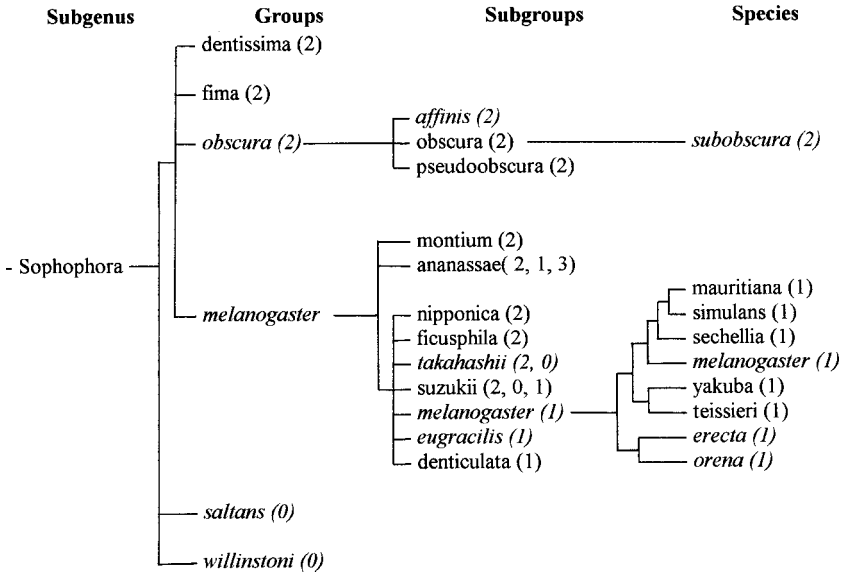


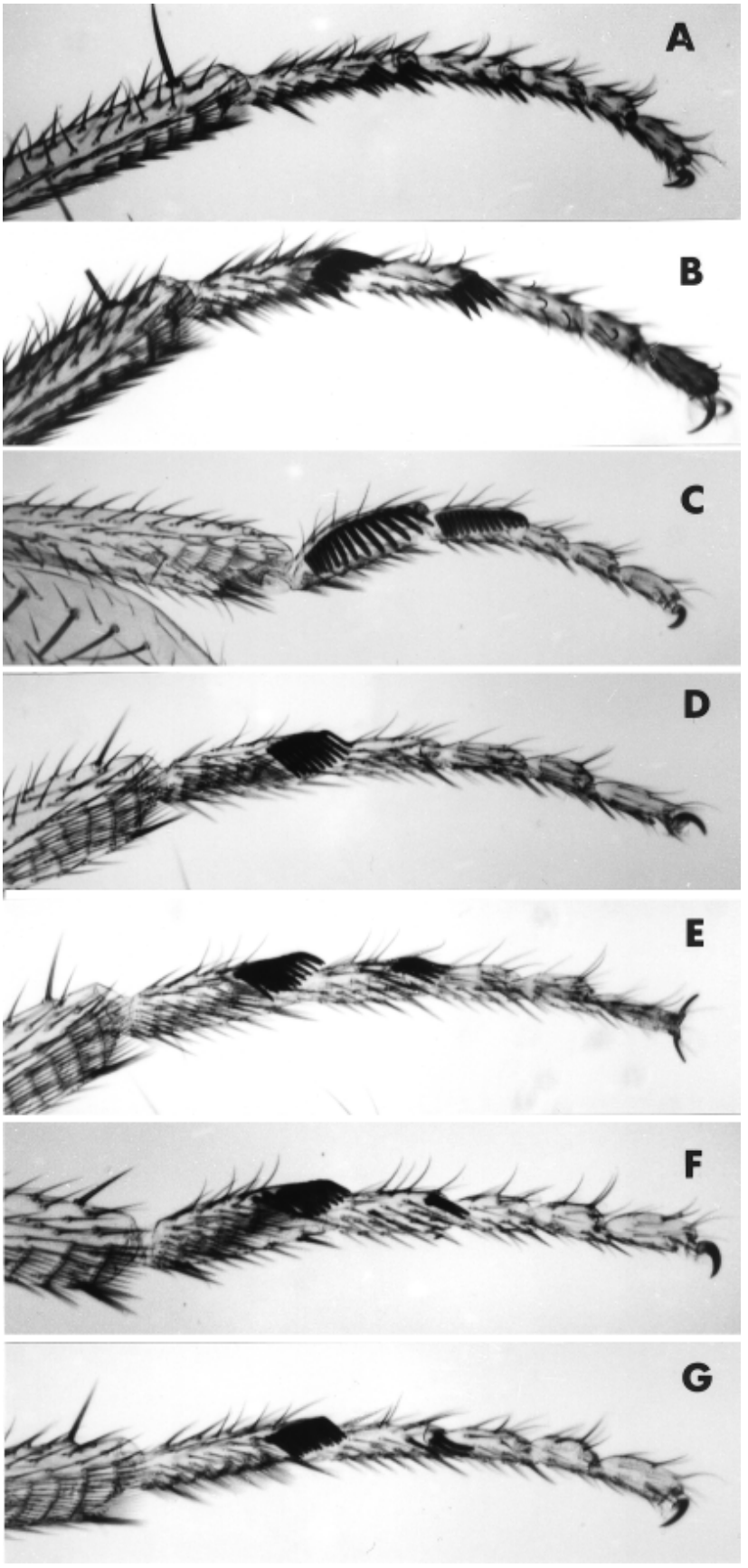
Fig. 3. Phylogenetic tree of the subgenus *Sophophora (Drosophila).* *In brackets number of tarsal segments with a sex comb. In bold letters species mentioned in the text.*

type clones in the anterior margin of scalloped wings of *Drosophila melanogaster* mutants: *Beadex* or *Lyra,* or also of *engrailed*[1] clones in the posterior margin, reveal the "Four row" pattern (García Bellido and Santamaría, 1972). The same "Four row" pattern is a character of other *Drosophila* species as *Drosophila affinis*. Both surfaces identical, each with a row of "stout" bristles and another row of chemosensory bristles is a plesiomorphic character shown for instance in *Musca*. Hackman and Väisänen (1985) consider that the intercalary migration of rows can be a general trend that accounts for this apomorphic character in the ventral surface of *melanogaster*. Two processes account for the present pattern of *Drosophila melanogaster,* versus a more ancestral pattern shown (García Bellido, 1983) by *Drosophila gibberosa* (Fig. 2C): the migration of rows and the dimorphism between monoinervated dorsal and ventral marginal rows. Two types of *Drosophila melanogaster* mutants restore the plesiomorphic pattern (2C): those of gain of function of *Dorsal wing (Dlw)* (Tiong *et al.,* 1995) and those of some *PcG* genes: *ph, Psc* and *Pc* (Denell and Frederics, 1983; Dura *et al.,* 1988; Adler *et al.,* 1991). Interestingly, clones of *trithorax (trx)*, a gene that antagonizes *PcG* genes, differentiate in the dorsal surface a pattern of bristles that mimics the ventral surface, and this is the phenotype of recessive, loss of function mutants of *Dlw.* Also, *Pc* mutants greatly enhance the gain of function phenotype of *Dlw*. Tiong *et al.* (1995) propose that genes of the *Polycomb* group act as negative regulators of *Dlw*$^{+}$.

The sex comb

We have studied another example, the sex comb phenotype of males (Santamaría, 1993; and Docquier, Randsholt and Santamaría unpublished results). This is a secondary sexual character, and it is expected for this type of traits to have a greater relationship with speciation mechanisms. The tarsal segments of the first leg bear an arrangement of round black bristles called the "sex comb". This sexual dimorphism of the foreleg is practically always present (Lemeunier *et al.,* 1986) in the species group (Fig. 3). The longitudinal alignment is an apomorphy derived from the transversal rows of more primitive flies in the *Drosophila* genus. The last two rows turn almost 90° (Hannah-Alava, 1958) to take up their present position in *Drosophila melanogaster*. In some species indication of a sexual difference is only the formation of heavily sclerotized bristles in the last transversal row of the first and second tarsii as in *Drosophila eugracilis* or *Drosophila takahashii* for instance (Bock and Wheeler, 1972 give precious descriptions and drawings). Thereby, most *sophophora* species have two sex combs, one in the first tarsus (or basitarsus) and another in the second. Some of them have also a sex comb in the third tarsus, other have only one sex comb in the first tarsus as it is the case in all species of the *melanogaster* subgroup (Fig. 4D). Others do not have a sex comb as it is the case of *saltans* and *willistoni.* These two groups, originated from the American Continent, differ markedly from the others (Throckmorton, 1975), after the Sophophora radiation in mid Oligocene or early Eocene (Lemeunier *et al.,* 1986). Among the other four groups (Fig. 3) the *obscura* group, with two sex combs is considered to be the most primitive (Hsu, 1949; Lakovaara and Saura, 1982; Lemeunier *et al.,* 1986). Interestingly, the mutant *scc* of *Drosophila subobscura* prevents the rotation and suppresses the differentiation of the first tarsal sex comb (Pascual and Mestres, 1995). This phylogeny (Fig. 3) suggests that the character "only one sex comb" is an apomorphy in the *melanogaster* subgroup, with respect to a more plesiomorphic "two sex combs". Interestingly, Stern (1954) used examples of species and mutants showing a sex comb in the second tarsus to argument about the concept of prepattern and states: "It seems possible that the evolutionary process which diversified the sex comb feature in different species began... with the response of mutated genes to preexisting developmental prepatterns".

Many mutations of *Drosophila melanogaster* cause the differentiation of two sex combs. Again, two groups of genes do this. In the PcG, mutants for *mxc, ph, E(z), Pc* and *crm* (Santamaría, 1993; Santamaría and Randsholt, 1995); the other group is heterogeneous and comprises alleles of *bric a brac "bab"* (Godt *et al.,* 1993) *sex comb distal "scd"* (Lindsley and Zimm, 1992) and *dachshund "dac"* (Docquier, Randsholt and Santamaría unpublished results). These convergent phenotypes suggest that probably the capability to develop a sex comb in the second tarsus exists in *Drosophila melanogaster* but is "repressed" in normal development. The *dac* gene has a role in eye development similar to that of *eyeless,* but probably acting downstream from it. The gain of function of *dac* in special circumstances can produce an ectopic eye. In the leg, the wild type product is expressed and necessary to give its identity to the femur, tibia and the three first tarsii (Lecuit and Cohen, 1997; Shen and Mardon, 1997). On the other hand, *bab* is expressed in the first, second, third and fourth tarsii. We have studied (Docquier, Randsholt and Santamaría unpublished results) dominant mutants of *dac* that should be classified as antimorphic (Muller, 1932) and that we called *dac Montiumlike (dac*^{Mtl}*)* because they mimic the phenotype of these species. The interactions between all these mutants (Docquier, Randsholt and Santamaría unpublished results) suggest that in the second tarsus of wild-type *Drosophila melanogaster*, a gene responsible for the sex comb differentiation is suppressed, but can be expressed because of different genetic alterations, bringing back the ancestral pattern, by



loss of function of *bab* or *scd*, or by the antimorphic activity of *dac*. Mutants for *mxc* or *crm,* that strongly enhance dac^{Mtl} and *bab,* should relax this repression as they relax HOM repression in other territories.

Conclusion

García Bellido and few others have provided a new perspective in the area of developmental biology when they assumed that limited numbers of simple constituents subjected to simple rules underlie morphogenesis. Should a process of development be controlled by many forces, it would be hopeless trying to have a holistic understanding. Evidently, an excessive reductionism is of non-avail when the processes are complex. It is possible now that Developmental Genetics and Evolution merge, that some simple examples of morphogenetic processes could be integrated in the understanding of how specie's characters evolve.

Pleiotropy of mutant phenotypes (see paper by J. Hodgkin in this issue) was often considered as a handicap to the understanding of the normal function of a gene, because it presupposes increased distance between primary cause and its consequences. Today, the perception of how developmental genetics could be integrated in the evolutive process, assesses pleiotropy as a positive character. I think that the interpretation given here about the pleiotropic phenotypes of PcG mutants has more than a heuristic value. If it is true that the causality of local alterations of the multimeric complex formed by PcG on evolutive processes would be difficult to prove, as buried in history, the test of the actual alteration of expression of, for instance *bab* or *dac* wild-type products in *Drosophila* species that show the same phenotype as the mutants, would open an experimental approach to prove or reject this hypothesis.

García Bellido (1983) has suggested that integrated patterns, that appear and disappear in far related species, may be due not to gradual selection, but to alterations of discrete combinations of cascades of gene activity. Saltational, versus more gradual or parcimonious evolutionary process can be explained by the changes brought about by alteration of the syntagmata controlled by *PcG* genes. Another interesting point would be that each integrated processes of morphogenesis is regulated from a spatio-temporal point of view. If those are constraints in normal development, they could be modulable variables of the evolution. The *PcG* genes could have been excellent tools to do this job. What distinguishes a species from another, even far apart, is less the differences in molecules than in organization.

Fig. 4. Pictures of the end of the tibia and tarsii of prothoracic legs. (A) *Drosophila takahashii.* **(B)***Drosophila subobscura.* **(C)** *Drosophila bocqueti (from the montium subgroup).* **(D)** *Drosophila melanogaster.* **(E)** *dacMtlT5, mutant of Drosophila melanogaster.* **(F)** *crm mutant of Drosophila melanogaster.* **(G)** *scd mutant of Drosophila melanogaster.*

"Colonization and specialization arrived by using differently the preexistent and preselected cascades of gene regulation. The potential of the regulatory processes of development used in evolution lies in the combinatorial nature of the regulatory networks" (Carroll, 1995). The recognized combinatorial activity of *PcG* genes make them specially prone to play an important role in the genesis and modification of living organisms.

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