The early Carboniferous one is even worse, without any fossil insects. But at the very end of this period and during the late Carboniferous, the insect diversity exploded, with a 'sudden appearance' of winged insects with very diverse feeding resources, e.g., carnivorous, plant suckers, leaf eaters, detritivorous, gall-makers, etc. The wingless clades remained a minority and the high diversification of the Carboniferous Hexapoda clearly concerned the winged forms. Wings and flight were probably the first crucial structures and function that allowed the first burst of diversification of the insects. Flight allows them to escape predators, find new resources, sexual partners, and travel to new environments. The most popular fossil insects are the Paleozoic 'giant' dragonflies Meganeuridae. These flying insects with very large wingspans (ca. 70 cm wide) had large bodies but comparable to those of some extant beetles. In fact, the unique really giant Carboniferous terrestrial arthropod was *Arthropleura*, a myriapod that was more than 1 m long. It is supposed that the great increase of oxygen proportion in the air during the Late Carboniferous favored the gigantism among the terrestrial arthropods, due to their breathing via trachea. The question is in fact more complex, because the winged insects knew a unique situation during the late Paleozoic, as they had no flying vertebrates as predators. As they were the only flying animals, they probably knew a phenomenon of parallel increases of sizes of predators (the Meganeuridae) and preys, the Palaeodictyoptera that also became larger and larger [2]. At the end of the middle Permian, both clades are very diverse, with still very large taxa, while the oxygen proportion began to decrease. The first gliding 'lizard-like' vertebrates are also recorded at the same time, and certainly began to predate these giant insects, which became rarer during the late Permian and no longer existed in the Triassic. The late Carboniferous was also the time of the oldest known holometabolous insects, with complete metamorphosis (wasps, beetles, scorpionflies), and of the oldest bugs (Hemiptera). These were discovered very recently because they were very small insects [3]. They are now the most diverse animal clades, with the 'big five' (Hemiptera, Hymenoptera, Diptera, Lepidoptera, and Coleoptera). But during all the Paleozoic, these insects were clearly very few. Holometaboly in itself was not 'sufficient' to cause their diversification and each of these orders 'separately' diversified during the last 220 Ma. The exact impact on the insects of the most important Permian–Triassic crisis of diversity remains difficult to estimate because there are very few latest Permian and earliest Triassic outcrops with insects. Thus if we know that the Triassic entomofaunas are very different from the Permian ones, we cannot establish that the great changes that occurred between the two periods happened during this crisis or before, during the late Permian or even at the end of the middle Permian. Nevertheless, the Palaeodictyoptera and the Meganisoptera are no longer present in the Triassic, while all the Triassic entomofaunas are clearly 'dominated' by the beetles and other Holometabola. Beetles were still minority during all the Permian in the fossil record. The 'true' flies (Diptera) and crown group of Hymenoptera are also dated from the Middle Triassic. At the end of this period, all the extant orders were present, except, maybe the parasite groups such as fleas (Siphonaptera), whose oldest fossils are middle Jurassic. The 'modern' entomofauna is thus much older than the extant mammal orders. During the Jurassic, the insects continued their diversification, with the first parasitoid wasps (there is no record of parasitoid insects before). The Cretaceous was the second crucial period for the insect (especially the Holometabola) diversification, with the oldest eusocial taxa (termites, wasps, bees, ants). The Albian–Cenomanian (ca. 100 Ma.) was the time of replacement of the gymnosperms by the angiosperms in all the terrestrial biotas, and the time of appearance of nearly all the extant insect families (even some extant genera have this age). It is also an important time of extinctions of several older Jurassic clades, replaced by extant taxa. Only the insects that adapted to the new environments related to flowering plants could diversify. The modern insect–plant relationships were established during the late Cretaceous. The recent new studies of the extraordinarily rich and diverse entomofauna of the 'mid' Cretaceous Burmese amber allowed one to discover that the Cretaceous insect world was as complex, rich and diverse as the extant one. The Cretaceous–Cenozoic (K–T) crisis had clearly a very weak impact on insect diversity, at least at the family level [4]. In fact, there were more extinctions and appearances of new families during the Paleocene–early Eocene than during the K–T crisis. These were periods of global warming followed by global cooling. The entomofaunas suffered the successive periods of cooling of the Oligocene, Miocene, and the Pliocene–Pleistocene glaciations, causing the extinctions of numerous widespread families that survived in small areas (the Australian mastotermitid termites or the Tasmanian hairy cicadid Tettigarctidae are the most spectacular examples).

The deep past history of insects is unique, with bursts of diversification ca. 330 Ma, 220 Ma, and 100 Ma ago. The causes of the first one remain poorly known, those of the second one are probably linked to the renewal of the ecosystems during the early Triassic, and the third one to the great floristic change. At least the K–T crisis did not affect much insect diversity. Thus the current crisis of biodiversity that begins to greatly affect the insect biomass, is extremely alarming. It may be more important than the K–T one.

Disclosure of interest The author declares that he has no competing interest.

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The metamorphosis of insects and their regulation

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Metamorphosis was a key innovation in insect evolution, wherein the individual acquires characteristic adult features and stops molting during postembryonic development. The ancestral metamorphosis mode was hemimetaboly, in which the embryogenesis gives rise to a first instar nymph with the essential adult body structure. The nymphs grow gradually and the final molt to the adult stage completes the formation of functional genitalia and wings. The metamorphosis mode known as holometaboly emerged from hemimetaboly, which is characterized by embryogenesis that produces a larva with a body form that may be substantially different from that of the adult. The larva grows through various stages until molting to the pupal stage, which bridges the gap between the morphologically divergent larva and that of the winged and reproductively competent adult. In the hemimetabolan and holometabolan modes, metamorphosis is regulated by two hormones: the juvenile hormone (JH) and the ecdysone, plus its biologically active derivative, 20-hydroxyecdysone (20E). 20E is a steroid, and its main role is to promote the successive molts, including the metamorphic one, whereas JH is a terpenoid, whose function is to repress metamorphosis [1]. The action of these hormones is underpinned by the mechanisms that transduce the hormonal signal through a pathway of gene activation. The 20E signaling pathway was first described in the 1990s [2], whereas the most important details of the JH pathway were unveiled recently. Important components of the JH signaling pathway are the JH receptor, which is the basic

helix–loop–helix-Per-ARNT-Sim (bHLH-PAS) protein known as methoprene tolerant (Met), to which JH binds, as unveiled by Jindra's group in the 2010 decade. Another important component is Taiman, also a bHLH-PAS protein that plays the role of co-receptor. Finally, the transcription factor Krüppel homolog 1 (Kr-h1) is the main transducer of the antimetamorphic signal of JH [1].

Krüppel homolog 1 *Kr-h1* was discovered in *Drosophila melanogaster* as a gene with structural similarity to the segmentation gene *Krüppel*, with which it shares the zinc-finger motifs and amino acid spacers connecting them. The first evidence that connected Kr-h1 and JH was also obtained in *D. melanogaster*. In this fly, the adult abdominal epidermis derives from larval histoblasts, which start proliferating after puparium formation. The experiments of Ashburner in 1970 showed that administration of JH prior to the prepupal stage prevents the normal differentiation of the abdominal epidermis, and the bristles that should be formed in the adult are shorter or lacking. In 2008, the experiments of Minakuchi and coworkers indicated that *Kr-h1* expressed ectopically in the abdominal epidermis during metamorphosis of *D. melanogaster* also resulted in missing or short bristles, thereby suggesting that Kr-h1 mediates the antimetamorphic action of JH. New experiments of Minakuchi and coworkers carried out in the beetle *Tribolium castaneum* in 2009 showed that RNAi depletion of Kr-h1 in young larvae caused a precocious larval–pupal transformation, providing clear evidence that Kr-h1 represses metamorphosis and works downstream from Met in the JH signaling pathway. The antimetamorphic action of Kr-h1 was generalized to hemimetabolans in two parallel papers published in 2011 and conducted, respectively, in the cockroach *Blattella germanica* by Lozano and Belles, and the bugs *Pyrrhocoris apterus* and *Rhodnius prolixus* by Konopová and coworkers. In these two studies, RNAi experiments showed that Kr-h1 depletion in nymphs in the penultimate or antepenultimate nymphal stage triggers precocious metamorphosis [1] (Fig. 1A).

E93 *E93* is an early gene in the ecdysone signaling cascade that is specifically expressed in late prepupae of *D. melanogaster*. As shown by the groups of Thummel and Baehrecke in the 1990 decade, the gene encodes for a protein with RHF domains significantly similar to pipsqueak motifs, which was found to be a key player in the degeneration process of the salivary glands during *D. melanogaster* metamorphosis. However, the action of E93 in metamorphosis is not restricted to the regulation of degeneration processes, given that it also plays morphogenetic roles. In 2012 Mou and coworkers observed that *E93* is widely expressed in adult cells of the pupa of *D. melanogaster*, where it is required for patterning processes. Studying the induction of the *Distal-less* (*Dll*) gene within bract cells of the pupal leg using epidermal growth factor (EGF) receptor signaling, Mou and coworkers found that E93 causes *Dll* to become responsive to EGF receptor signaling, thus indicating that E93 is both necessary and sufficient for determining this switch. These results suggested that E93 controls the responsiveness of many other target genes and that it is generally required for patterning during metamorphosis. Subsequent RNAi experiments reported by Ureña and coworkers in 2014 showed that E93-depleted *D. melanogaster* larvae are able to pupate but die at the end of the pupal stage. In *T. castaneum*, E93 depletion by RNAi prevented the pupal–adult transition, resulting in the formation of a supernumerary second pupa. Similar results were obtained in the cockroach *B. germanica*, where E93 depletion in nymphs prevented the nymphal–adult transition, giving rise to repeated supernumerary nymphal instars (Fig. 1B). The same year, Belles and Santos showed that the expression of *E93* in juvenile nymphs of *B. germanica* is inhibited by the transcription factor Kr-h1, thus uncovering the essential mechanism by which JH represses metamorphosis, which was named MEKRE93 pathway [3].

The MEKRE93 pathway in hemimetabolan species The observation that Kr-h1 represses *E93* expression led to propose the MEKRE93 pathway as the essential axis regulating insect metamorphosis. Accordingly, in nymph–nymph transitions, JH acts through its receptor Met-Taiman to induce the expression of *Kr-h1*, while Kr-h1 represses the expression of *E93*. In contrast, the decline of JH production in the final juvenile stage interrupts *Kr-h1* expression, *E93* becomes de-repressed, thus triggering adult morphogenesis (Fig. 2A) (Belles and Santos, 2014). RNAi experiments in *B. germanica* by the same authors also revealed that E93 depletion increases *Kr-h1* expression, thus indicating that *Kr-h1* and *E93* are reciprocally repressed. The inhibitory action of Kr-h1 upon *E93* expression was corroborated two years later in the holometabolan *T. castaneum* by Ureña and coworkers, which extended the MEKRE93 pathway framework to holometabolan metamorphosis.

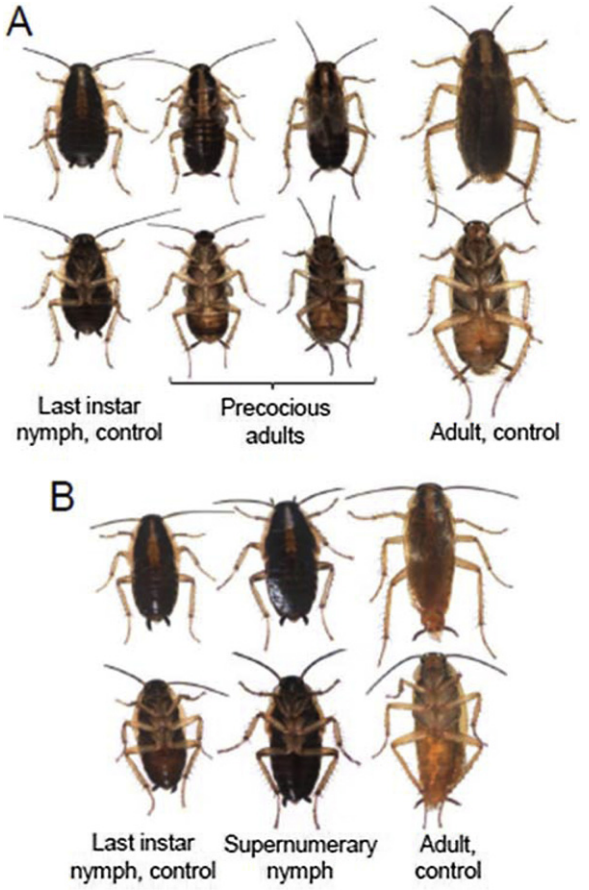

Fig. 1 Regulation of metamorphosis by Krüppel homolog 1 (Kr-h1) and E93, exemplified by the cockroach *Blattella germanica*. A. Depletion of Kr-h1 in penultimate nymphal instar triggers a precocious metamorphosis at the next molt, thus a miniature adult is produced instead of a last instar nymph. B. Depletion of E93 in last nymphal instar inhibits metamorphosis, thus a supernumerary nymphal instar is produced instead of an adult. See the text for additional information and sources.

The MEKRE93 pathway in holometabolan species The main difference between the hemimetabolan and holometabolan metamorphoses is the regulation and function of the Broad complex (BR-C) zinc-finger transcription factors. In hemimetabolan species, BR-C is mainly involved in promoting the growth of wing primordia. For example, this was shown in *B. germanica* by Huang and coworkers in 2013, who additionally reported that *BR-C* expression is induced by JH and Kr-h1 during juvenile stages. One year later, Ureña and coworkers found that BR-C is repressed by E93 in the metamorphic transition. Furthermore, RNAi studies made in 2019 by the group of Mito and Noji in the cricket *Gryllus bimaculatus*, a hemimetabolan species, confirmed the mentioned

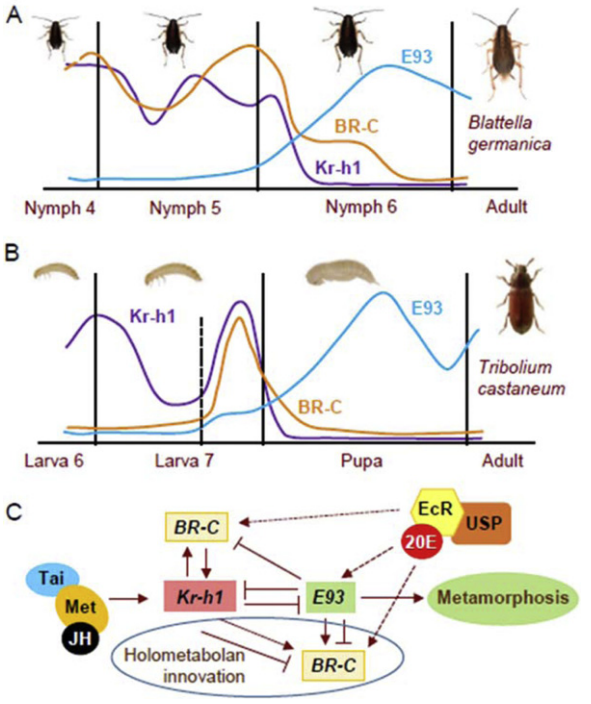

Fig. 2 The MEKRE93 pathway. A. Expression patterns of Krüppel homolog 1 (Kr-h1), E93 and Broad complex (BR-C) in hemimetabolan insects (*Blattella germanica*). B. Expression patterns of Kr-h1, E93 and BR-C in holometabolan insects (*Tribolium castaneum*). C. The MEKRE93 pathway in hemimetabolan and holometabolan species. See the text for additional information and sources.

interactions, and additionally discovered that BR-C and Kr-h1 are reciprocally activated. In sharp contrast, and as shown mainly by the group of Riddiford in the decade of 1990, BR-C triggers the formation of the pupal stage in holometabolan species, where JH inhibits the expression of *BR-C* during larval stages and stimulates *BR-C* expression after pupal commitment (Fig. 2B). In 2019, Chafino and co-workers showed that E93 is involved in triggering the pupal stage, as it promotes *BR-C* expression in *T. castaneum*. The whole data indicates that the MEKRE93 pathway is conserved in the holometabolan species, which added the E93/BR-C interaction loop to the ancestral (hemimetabolan) pathway during the evolutionary transition from hemimetaboly to holometaboly (Fig. 2C).

Disclosure of interest The author declares that he has no competing interest.

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Evolution of aposematism and mimicry in butterflies: Causes, consequences and paradoxes

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Insects represent valuable food for many predators, and as such they have evolved a large panel of anti-predator adaptations. While deceptive adaptations such as camouflage and masquerade rest on avoiding detection by predators, aposematism relies on advertising chemical defenses with conspicuous warning signals, such as colorful patterns. Because the efficiency of a warning signal increases with its own local abundance, multiple aposematic prey exposed to the same predators benefit from converging on the same warning signal, a phenomenon originally observed by Henri Bates and Alfred Wallace and later understood and formalized by the German naturalist Fritz Müller [1] and called Müllerian mimicry. Convergence in warning signal is therefore due to positive frequency-dependent selection, leading to a 'strength in numbers' effect. Species sharing the same warning are said to be co-mimetic and interact mutualistically (i.e. individuals from either species benefit from the presence of individuals of co-mimetic species), and form mimicry rings.
Müllerian mimicry exists in a variety of organisms, including frogs, wasps, millipedes and beetles, but it has been best studied in butterflies (Fig. 1). Two neotropical butterfly clades have attracted considerable attention: the genus *Heliconius* (43 species) and the tribe Ithomiini (393 species).
Here, I review recent genetic and ecological results on *Heliconius* and Ithomiini butterflies that advance our knowledge on the proximal and ultimate drivers of mimicry, and on the evolutionary and ecological consequences of mimicry in terms of speciation, genetic architecture and ecological niche evolution. I also present recent results that help us understanding two apparent paradoxes: the embarrassing diversity of mimicry patterns despite strong selection for convergence, and the evolution of transparent wing patterns in aposematic butterflies, where conspicuous signals are supposed to be favored.

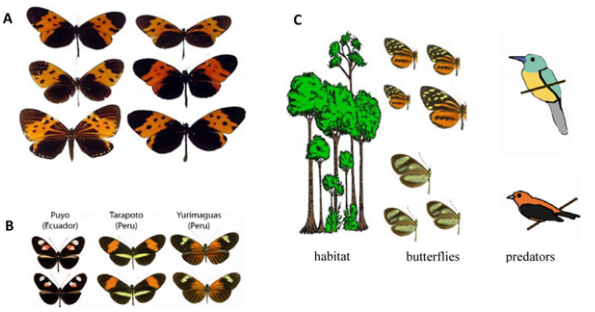

Fig. 1 A. A common mimicry ring in the Andean foothills. Left column, from top to bottom: *Hypothyris mansuetus* (Nymphalidae: Ithomiini), *Hyposcada anchiala* (Nymphalidae: Ithomiini), *Chetone* sp. (Erebidae: Arctiinae). Right column, from top to bottom: *Mechanitis messenoides* (Nymphalidae: Ithomiini), *Heliconius numata* (Nymphalidae: Heliconiini), *Melinaea mothone* (Nymphalidae: Ithomiini). Photo credit: Mathieu Joron. B. Co-mimetic subspecies of *Heliconius melpomene* (top) and *H. erato* (bottom) in three different regions of their common range, showing geographic variation in wing colour pattern. Photo credit: Jim Mallet. C. An illustration of microhabitat segregation of predators and mimicry rings. Illustration credits: Nicolas Chazot (trees) and Marianne Elias (birds); photo credits: Keith Willmott.