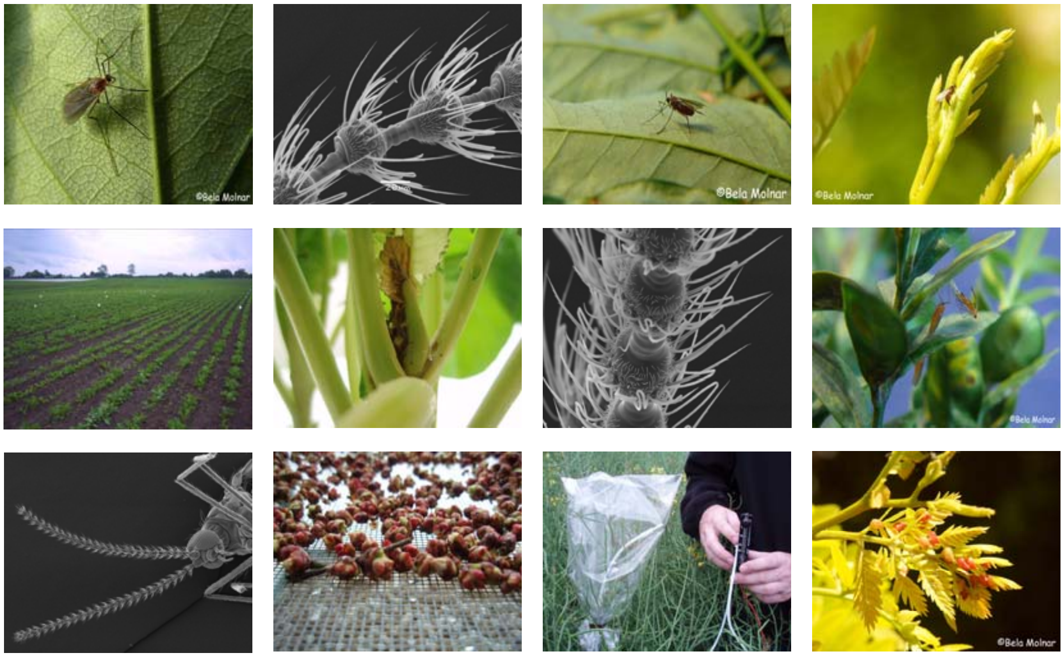
EVOLUTION AND SPECIATION

– INSECTS AS MODEL ORGANISMS

Tina Boddum

Introductory Paper at the Faculty of Landscape Planning, Horticulture and Agricultural Science 2008:3
Swedish University of Agricultural Sciences
Alnarp, November 2008



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ISSN 1654-3580

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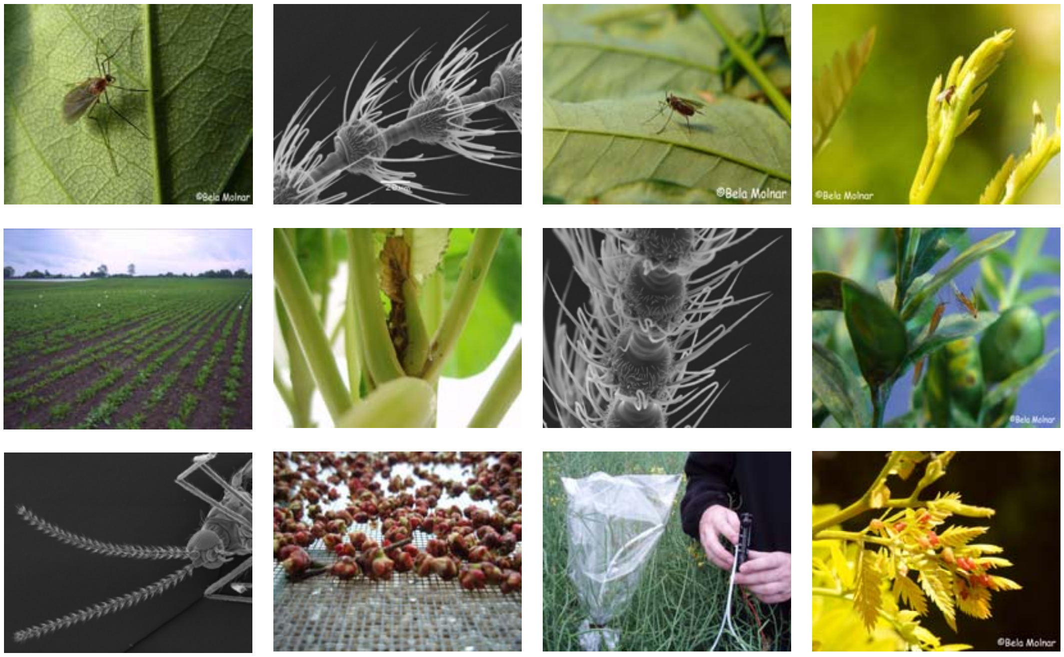

"Nothing in biology makes sense except in the light of evolution"

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1. Preface

This introductory paper is part of my PhD project: “Host shift induced speciation in gall midges”. The project is conducted at the Swedish University of Agricultural Sciences (Alnarp), department of Plant Protection Biology, division of Chemical Ecology. The project is part of IC-E3, supported by a Linnaeus Grant (Formas, Sweden). My supervisors are Ylva Hillbur, Bill S. Hansson and Göran Birgersson. The first part of this paper is a general overview of evolution and speciation. This is followed by a more detailed part about insects and their speciation. At the end of the paper the gall midges (Diptera: Cecidomyiidae) are introduced.

2. Summary

Charles Darwin is the father of evolution as we know it today. In his book “The origin of species” he states that new species originate from ancestral species that change over time, and that the mechanism of the change is natural selection. How the variation natural selection need is generated and passed from generation to generation was solved by Gregor Mendel and Thomas Hunt Morgans (and his group). Based on experiments with pea plants, Mendel formed laws about segregation and assortment of traits and Morgans group demonstrated that Mendels hypothetical factors are specific points on the chromosome.

Evolution mostly deals with how populations become adapted to their environment, but not how this adaption leads to speciation. For speciation to occur, barriers for the gene flow between populations have to evolve. There are two general modes of speciation defined by how the gene flow between populations is interrupted. In allopatric speciation a physically barrier isolates a population, whereas sympatric speciation occur within a single geographical area and reproductive isolation arises between individuals that always have the opportunity to interbreed.

Insects are good models when the mechanisms underling evolution and speciation are studied, there are more than one million species and their diversity and distribution is amazing. Olfaction is the primary sense by which the environment is interpreted by insects, and olfactory cues can be

important for separation of population evolving in sympatry. That was demonstrated by Löfstedt and co-workers who studied nine species of sympatric ermine moth *Yponomeuta*. All species had a mixture of (*E*)-11 and (*Z*)-11 tetradecenyl acetate as primary pheromone compounds, however, the females produced the compounds in a specific ratios that never overlapped if the species were not isolated by other barriers.

Pheromones are well studied compared to the plant-produced odors. However, insects can detect relevant plant odors with the same selectivity and sensitivity as they detect pheromones. The number of volatiles emitted from fruit and plants is much higher than the number of components in the female pheromone, yet, Stensmyr et al. (2003) demonstrated that *Drosophila melanogaster* only needs a few key components to locate and detect a food source.

The *Rhagoletis pomonella* sibling species complex is a model system for sympatric host race formation and speciation (e.g. Forbes et al., 2005; Linn et al., 2003; Linn et al., 2005b). The complex consists of several strains with different host preference – a preference based on olfactory cues. Host choice is of evolutionary significance for *Rhagoletis* as they mate on or near the fruit of their respective host plant. Adult flies tend to mate on or near the same species of host fruit as the one they infested as larvae. Thus, differences in host preferences can translate into mate choice and can act as pre-mating barriers to gene flow.

As for other insects, gall midge behavior has been shown to be guided by olfactory cues; they use pheromones when locating a suitable mate and plant volatiles for host plant recognition. Thus, host plant volatiles might be important when gall midges shift between hosts and subsequently in the formation of new gall midge species. In my thesis I will study possible evolutionary mechanisms behind the great diversity of the gall midges. The two main questions I will address in my thesis are: do gall midges associated with the same host plant use the same or a similar set of odors to identify it? And, conversely, do closely related species that have different host plant requirements respond to odors common for the different plants?

3. Evolution

Evolution is one of the most unifying concepts in biology, as any aspect of an organism – from mating behaviour to the mode of photosynthesis or a mutation in a gene – can be explained from an evolutionary perspective (Grimaldi and Engel, 2005).

3.1 The ideas

Charles Darwin (1809-1882) is the father of evolution as we know it today (Campbell et al., 1999a; Horan, 2006). His book “The origin of species” is the basis for modern evolution and speciation theories. Darwin proposed that new species originate from ancestral species that change over time. He added the mechanism of evolutionary change – natural selection (Freeman and Herron, 2004; Grimaldi and Engel, 2005). Alfred R. Wallace (1823-1913) had the ideas independent of Darwin, and was co-author with Darwin on the original paper proposing natural selection as the mechanism behind evolution (Freeman and Herron, 2004; Grimaldi and Engel, 2005).

Darwin’s theory is based on four postulates from the introduction to “The Origin of species” (Table 1; Darwin, 1859). Darwin regarded life in nature as a competition, where the fittest individuals win. The fitness of an individual refers to how well it survives and reproduces compared to other individuals in the population. Traits that increase the fitness of an organism relative to individuals without those traits makes it better adapted (Campbell et al., 1999a; Freeman and Herron, 2004). However, Darwin could not explain how variation was passed from generation to generation and how it was generated. That was solved by Gregor Mendel (1822-1884) and Thomas Hunt Morgan’s (1866-1945) group at Columbia University. Based on experiments with pea plants, Mendel formed laws about segregation and assortment of traits (Freeman and Herron, 2004; Grimaldi and Engel, 2005) and Morgan’s group demonstrated that Mendel’s hypothetical factors are specific points on the chromosome (Allen, 1985a; Allen, 1985b).

Table 1. Darwin’s original postulates about evolution as stated in “the Origin of species” and the postulates re-stated after the Modern Synthesis (Freeman and Herron, 2004).

Darwin’s postulates in the “the Origin of species”	**Re-statement after the Modern Synthesis**
1. Individuals within populations are variable	1. As a result of mutations creating new alleles, and segregation and independent assortment shuffling alleles into new combinations, individuals within populations are variable for many traits
2. The variations among individuals are, at least in part, passed from parents to offspring	2. Individuals pass their alleles to their offspring intact
3. In every generation, some individuals are more successful at surviving and reproducing than others	3. In every generation, some individuals are more successful at surviving, and reproducing than others
4. The survival and reproduction of individuals are not random; instead they are tied to the variation among individuals. The individuals with the most favorable variations, those who are better at surviving and reproducing, are selected for.	4. The individuals that survive and reproduce, or reproduce the most, are those with the alleles and allelic combinations that best adapt them to their environment.

In the 1920s Darwinian selection and Mendelian inheritance were integrated into the Modern Evolutionary Synthesis by Dobzhansky, Mayr, Simpson and Stebbins (Campbell et al., 1999b; Grimaldi and Engel, 2005; Freeman and Herron, 2004; Table 1). The synthesis emphasizes the importance of populations as the unit of evolution; it states that mutations are the source of raw material for evolutionary change, that natural selection is the most important mechanism of evolution, and that large changes can evolve as accumulation of small changes occurring over long periods of time.

The classical Darwinian theories and the Modern Synthesis are now challenged, but these theories have shaped most current ideas about evolution.

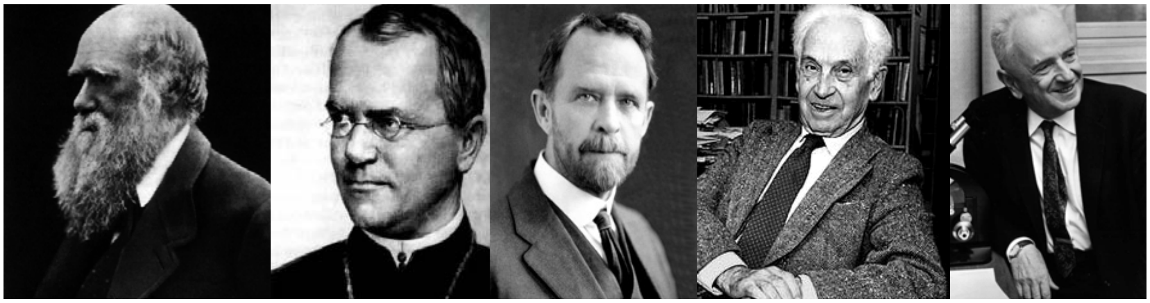


Figure 1. Some of the great thinkers in the history of evolution and speciation (from left to right): Charles Darwin (1809-1882), Gregor Mendel (1822-1884), Thomas Hunt Morgan (1866-1945) Ernst Walter Mayr (1904-2005, Theodosius Grygorovych Dobzhansky (1900-1975).

3.2 Level of selection

A central evolutionary concern is what *unit* is actually selected (Campbell et al., 1999a; Dawkins, 1976; Jablonka and Lamb, 2006). There are two general ways of looking at natural selection from: the gene's angle and that of the individual. The classical way is focusing on the individual, but Dawkins (1976) introduced the gene's view of nature. Dawkins argued that genes and not the whole organism is the unit of natural selection. The organisms are just “survival machines” for the genes. However, the strictly gene-centered concept of natural selection is maybe also too simplistic, and the two ways are probably equivalent (Freeman and Herron, 2004; Jablonka and Lamb, 2006). Natural selection acts on phenotypes, but for evolution to occur there must be genetic variation that natural selection can act on.

3.3 More than genes?

The phenotype of an individual is traditionally regarded as the summation of two totally independent factors: the genes and the environment (Jablonka and Lamb, 2006). However, after the introduction of epigenetic variation the separation is less clear. Epigenetic variation is inherited variation that is sensitive to environmental input (Jablonka and Lamb, 2006; Lindqvist et al., 2007; Richards, 2006). Traditionally, it was believed that inherited information only changes at random and without direction towards a particular phenotypic outcome, but recent findings indicate that the environment can affect the genotype (Hoy, 2003). The most well known example is transposable elements. Transposable elements are elements (with an RNA or DNA intermediate) that can move from site to site in the genome (Hoy, 2003). The activity of the elements can be induced by environmental factors, especially stress (Capy et al., 2000). This suggests that transposable elements can create new genetic variation that is useful under conditions where the fitness of an organism is reduced (Capy et al., 2000; Hoy, 2003). The evolutionary significance of epigenetic mechanisms was first discovered in plants where the adaptive significance is clear (Jablonka and Lamb, 2006). Plants cannot avoid harsh conditions by moving away and epigenetics might allow them to respond in another way (Jablonka and Lamb, 2006).

4. Speciation

Despite the title of his book, Darwin devoted little space for the origin of species (Campbell et al., 1999a; Coyne, 1994). He concentrated on how populations become adapted to their environment through natural selection, but not how this adaption leads to speciation (Campbell et al., 1999a; Coyne, 1994). Now, the study of speciation is one of the most active areas of evolutionary biology, and progress has been made in documenting and understanding phenomena in all aspects of speciation (Turelli et al., 2001). However, there is a fundamental problem in the field. It is very difficult to define exactly what "species" is. "*It is as if on one hand we know just what "species" means, and on the other hand, we have no idea what it means*" (Hey, 2001)

The idea of organic discontinuity has a long tradition, beginning with Linnaeus' classification (Coyne, 1994). The clustering of organisms into discrete groups (i.e. species) can be seen both in morphology, gene sequences and reproductive compatibility (Turelli et al., 2001). However, some biologists argue that the discontinuities are artefacts of human perception (Coyne, 1994), and in some groups e.g. in plants and asexually reproductive taxa, it is difficult to separate different species (Coyne, 1994; Turelli et al., 2001).

But why will organisms cluster into groups separated by gaps? And what properties of sexually reproducing organisms and their environment lead to the evolution of discrete species? Two (not mutually exclusive) explanations exist: the “ecological explanation” and the “sexual isolation explanation” (Coyne and Orr, 2004; Turelli et al., 2001). The ecological explanation states that ecological niches are discrete and that the clusters result from the ways different species exploit physical resources. Furthermore, disruptive selection (Figure 2D) makes hybrids that “fall between niches" less fit. The sexual isolation explanation states that groups will adapt different to the environment. Over time the number of differences will increase (divergent evolution) and result in the formation of new species (Coyne and Orr, 2004).

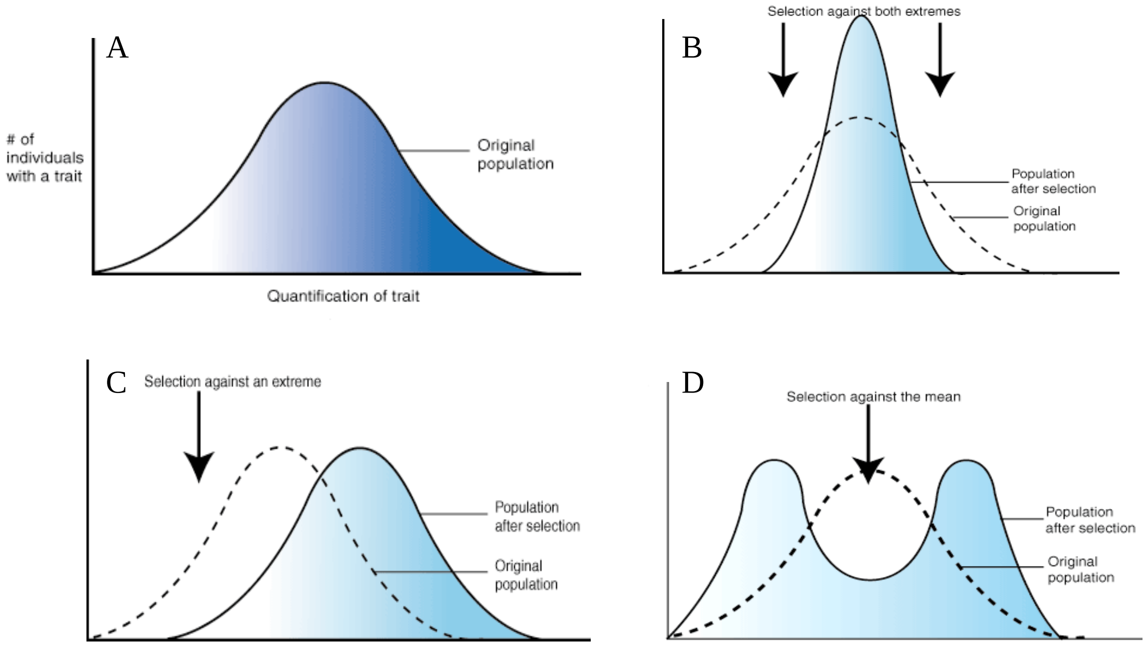


Figure 2. Three general modes of selection. A) The original population. B) Stabilizing Selection: Intermediate traits are favored by selection, resulting in a decrease in variation. C) Directional Selection: One extreme trait is favored, resulting in a change in the mean value of the trait. D) Disruptive Selection: Extreme traits are favored over the intermediate trait values, can divide the population into two distinct groups. Disruptive selection plays an important role in speciation (http://www.sparknotes.com/).

4.1 Species concepts

The biological species concept is a classical and widely accepted species concept (Berlocher, 1998; Campbell et al., 1999b). It defines a species as a group of actually or potentially interbreeding populations that are reproductively isolated from other such groups (i.e. they have the same gene pool). New species arise when the evolution of reproductive isolation mechanisms prevents gene exchange between populations (Turelli et al., 2001, Campbell et al., 1999c). Population biologists are discovering more and more cases where the biological species concept is not valid e.g. in asexual organism where the concept of breeding does not make sense. That results in the development of several other species concepts (Box 1) (Campbell et al., 1999a; Coyne, 1994; Coyne and Orr, 2004; Grimaldi and Engel, 2005).

Box 1. The biological species concept and some proposed alternatives (Campbell et al., 1999a; Coyne, 1994; Coyne and Orr, 2004)

Biological species concept**:** Emphasizes reproductive isolation. Species are groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups

Cohesion species concept: Focuses on mechanisms that maintain species as discrete phenotypic entities. Each species is defined by its complex of genes and set of adaptations. Applicable to organisms that reproduce without sex

Ecological species concept**:** Defines species on the basis of where they live and what they do

Evolutionary species concept**:** A species is a single lineage of ancestral and descendant populations that are evolving independently of other such groups.

Genotypic cluster species concept**:** A species is a (morphologically or genetically) distinguishable group of individuals that has few or no intermediates when in contact with other such clusters

Morphological species concept: Defined species by measurable anatomical differences (morphological criteria). It is practical to apply in the field, even to fossils.

Phylogenetic species concept**:** A species is the smallest monophyletic group of common ancestry

Recognition species concept: Emphasizes mating adaption's that become fixed in a population as individuals "recognize" certain characteristics of suitable mates

4.2 Isolation of populations

Speciation in sexually reproductive organisms is based on the evolution of reproductive barriers for the gene flow between populations (Campbell et al., 1999b; Turelli et al., 2001). Barriers can occur before mating, between mating and fertilization, or after fertilization (Figure 3). Prezygotic barriers occur before fertilization (figure 3) (Campbell et al., 1999b; Coyne and Orr, 2004). A common prezygotic barrier is habitat isolation, where a geographical barrier (e.g. flooding) can divide a population into several isolated populations (Campbell et al., 1999b)

Postzygotic barriers exercise isolation after fertilization (Figure 3; Table 2). The isolation can be divided into extrinsic postzygotic and instrinsic postzygotic (Campbell et al., 1999b; Coyne and Orr, 2004; Turelli et al., 2001). In extrinsic postzygotic isolation, hybrids are unfit because they "fall between" parental niches as they have an intermediate phenotype that is less fit (Coyne and Orr, 2004). In intrinsic postzygotic isolation, hybrids suffer developmental defects that make them unable to survive or develop normally (Coyne and Orr, 2004).

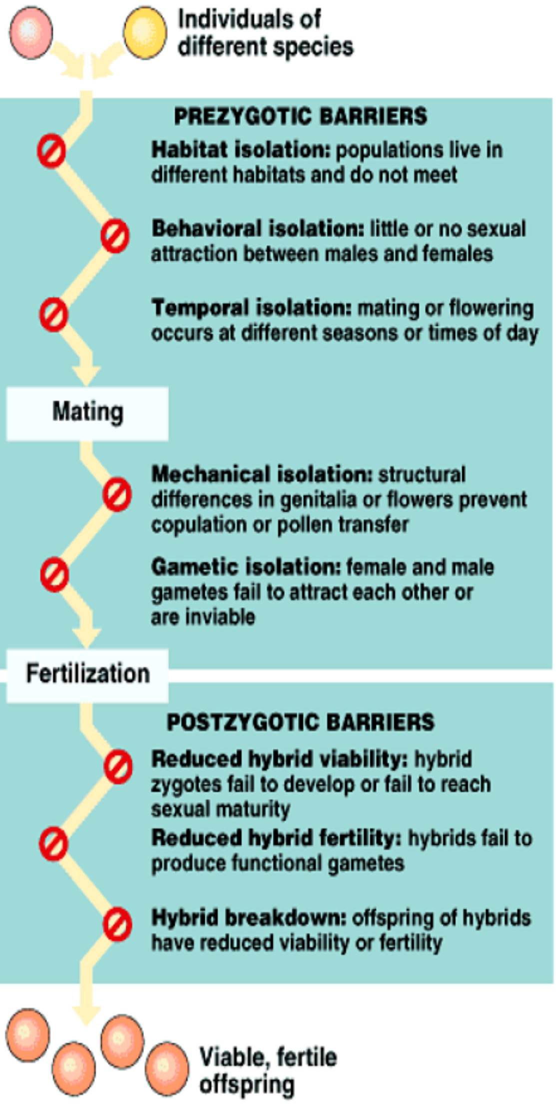


Figure 3. The reproductive barriers that prevent gene flow between two different species. Prezygotic barriers occurs before mating, while postzygotic do after (Campbell et al., 1999b).

Table 2. Classification of postzygotic reproductive isolation (Coyne and Orr, 2004)

Extrinsic

Ecological inviability: Hybrids develop normally but suffer decreased viability, as they cannot find a suitable ecological niche

Behavioral sterility: Hybrids have normal gametogenesis but suffer lowered effective fertility because they cannot find mates. Hybrids might have an intermediate courtship behavior or other phenotypes that render them unattractive to individuals of the opposite sex.

Intrinsic

Hybrid inviability: Hybrids have developmental defects causing full or partial inviability.

Hybrid sterility:

- **Physiological sterility:** Hybrids suffer developmental defects in their reproductive system causing full or partial sterility.
- **Behavioral sterility:** Hybrids suffer a neurological defect that renders them fully or partially incapable of courtship

4.3 Types of speciation

There are two general modes of speciation: allopatric speciation and sympatric speciation (Figure 4). They are defined by how the gene flow among populations is interrupted. In allopatric speciation a geographical barrier physically isolates a population and initially blocks gene flow, whereas in sympatric speciation intrinsic factors e.g. chromosomal changes or nonrandom mating alter the gene flow.

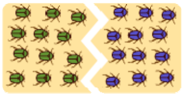
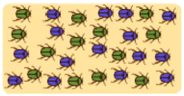
Mode of speciation	New species formed	
Allopatric allo = other, patric = place	From geographically isolated populations	
Sympatric sym = same, patric = place	within the range of the ancestral population	

Figure 4. The two general modes of speciation. Top) allopatric speciation. Bottom) sympatric speciation

4.3.1 Allopatric speciation

In allopatric speciation populations are separated by geographical isolation. In allopatric speciation extrinsic factors – as great distance or a physical barrier – prevents two or more groups from mating (Campbell et al., 1999b). Physical isolation is an effective barrier to gene flow and in many cases it is an important trigger for divergence. When no forces impose reproductive capability between isolated populations the populations will, given enough time, become incompatible (Turelli et al., 2001). Allopatric speciation is most likely to occur if a small population in the periphery of a species' range gets isolated. The individuals in the periphery are often extremes with a gene pool that differs from that of the rest of the population (Campbell et al., 1999b; Freeman and Herron, 2004). In a small population random mutations or new combinations of existing alleles with neutral adaptive value may get fixed by chance and evolution by natural selection may be different than in the parent population (Campbell et al., 1999b).

4.3.2 Sympatric speciation

Since the nineteenth century it has been debated if speciation requires geographical isolation (Berlocher, 1998). The authorities (e.g. Mayr and Dobzhansky) argued that geographic isolation is a necessary first step for divergence in animals whereas Guy Bush emphasized ecological adaption as an important factor in speciation (Bush, 1998; Feder et al., 2005). Sympatric speciation is still questioned and recent analyses show that allopatric speciation is the most common mode (Barraclough and Nee, 2001).

Two central factors differ between sympatric and allopatric speciation. Firstly, sympatric speciation does not require large-scale geographic distance to reduce gene flow between parts of a population (Campbell et al., 1999b; Freeman and Herron, 2004). Instead new species arise within the range of the parent population as the result of reproductive barriers between the mutant and the parent populations. Secondly, in sympatric speciation gene flow may continue for a number of generations after the populations have become separated, whereas complete isolation arises between populations evolving in allopatry.

A four stage series has been proposed for sympatric speciation via host plant shift for phytophagous insects (Berlocher, 1998): (1) partially reproductively isolated host races (2) species isolated only by host fidelity (3) species with partial prezygotic and/or postzygotic isolation unrelated to host fidelity and (4) totally reproductively isolated species.

4.4 Phylogenetic relationship

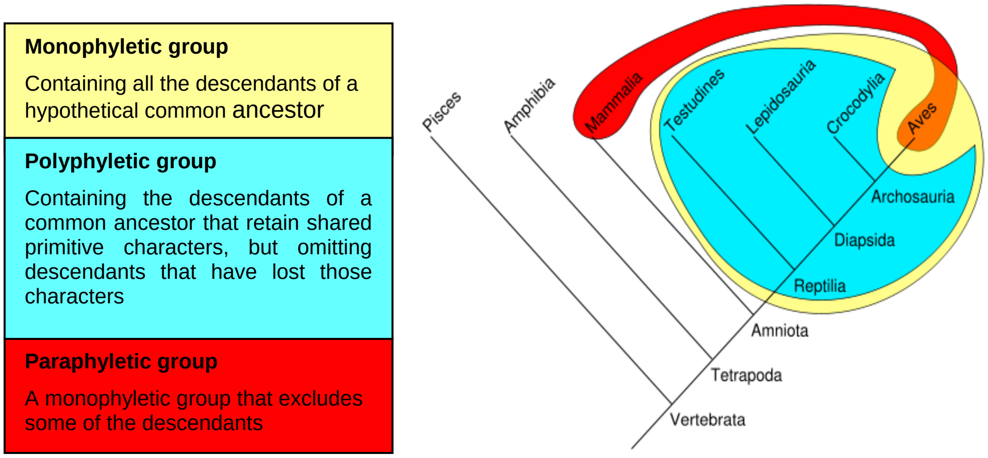
Phylogenetic classification is the most useful type of systematics (Grimaldi and Engel, 2005). Organisms are analyzed and divided into a hierarchical pattern (a cladogram or phylogenetic tree) based on homologies in behavior, morphology or molecular traits (Box 2; Hoy, 2003; Schoonhoven et al., 2005)

***Plesiomorphi**:* similarities that arose in a distant common ancestor (ancestral or “primitive”)

Apomorphic: similarities that arose in a resent common ancestor (derived or “advanced”)

Box 2. Classification of characteristics (Grimaldi and Engel, 2005; Hoy, 2003)

Phylogenetic classification allows interpretation of evolutionary patterns e.g. explanations for creation and termination of lineages (Grimaldi and Engel, 2005). Species can be divided into monophyletic, polyphyletic and paraphyletic groups based on the associations of their ancestors (Figure 5), however, classification must be strictly monophyletic to have any explanatory power (Grimaldi and Engel, 2005).



Monophyletic group

Containing all the descendants of a hypothetical common ancestor

Polyphyletic group

Containing the descendants of a common ancestor that retain shared primitive characters, but omitting descendants that have lost those characters

Paraphyletic group

A monophyletic group that excludes some of the descendants

Figure 5. Phylogenetic classification, species are divided into monophyletic, polyphyletic and paraphyletic groups.

5. Insects as models in evolution

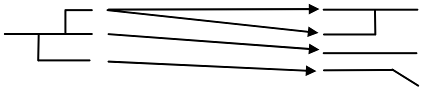
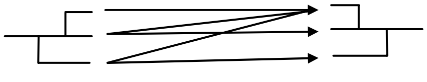
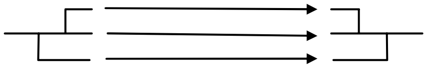
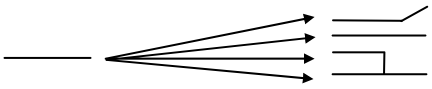
Insects were among the first animals on land, and the diversity and distribution of now living insects is astonishing. With one million species, insect are the most diverse organisms in the history of life – both in numbers of species and variety of structures and behaviors (Grimaldi and Engel, 2005; Schoonhoven et al., 2005)

5.1 Plant insect interactions

Several hypotheses that explain the diversity of herbivorous insects have been proposed (Schoonhoven et al., 2005). One theory is that herbivorous insects and their host plants are involved in “an arms race” through reciprocal evolution/co-evolution. The first plants are older than the first insects, but the currently largest group of plants – the angiosperms – evolved in the Cretaceous period where insects were abundant. It is, however, debated if the plants are affected by the herbivorous insects or if the insects just follow the evolution of the plants.

The evolution of host-plant choice can be illustrated with cladograms showing the correlation between insect and host-plant phylogenies (Table 3; Schoonhoven et al., 2005).

Table 3. Four types of cladogram illustrations of the divergence of existing plant and insect species from their ancestors are found. Type B and C suggest polygenetic conservatism – that speciation in herbivorous insects is often accompanied by shifts between closely related plant taxa.

Type	Insects	Specificity of insects	Host plants	Cladogram: Insects	Plants
A	Closely related	Oligophagous/monophagous	Distantly related		
B	Closely related	Oligophagous/monophagous	Closely related		
C	Closely related	Monophagous	Closely related		
D	One species	Polyphagous	Distantly related		

6. Examples of insect evolution and speciation

6.1 Sex pheromones and reproductive isolation in moths

Species-specific sex pheromones can provide reproductive isolation in moths. The specificity of the sex pheromone is achieved by specific compounds or by a specific ratio of compounds (Hansson, 1995). The pheromone is typically produced and released by the female with males of the same species perceiving the pheromone and flying upwind to the female (Karlson and Lüscher, 1959; Linn and Roelofs, 1995).

The evolution of the complex pheromones might be the result of the requirement for a distinctive signal in an environment where several species use the same or similar compounds (Linn and Roelofs, 1989; Löfstedt, 1993). Insects might show varying degrees of specificity depending on the contact with closely related species (Linn and Roelofs, 1989; Löfstedt, 1993).

Löfstedt and co-workers examined nine species of the small ermine moth *Yponomeuta* living sympatrically in Europe (Löfstedt and Herrebout, 1988; Löfstedt and Vanderpers, 1985). All species had a mixture of (*E*)-11 and (*Z*)-11 tetradecenyl acetate as primary pheromone compounds. The females produced the compounds in specific ratios, however, some species produced the same ratio (Figure 6) (Löfstedt, 1986; Löfstedt et al., 1991). Nevertheless, the range did only overlap for species that were isolated by other barriers e.g. lived on different host-plant, was temporally separated or had an additional pheromone component.

The pheromone (and the capacity to respond to it) is directly associated with reproductive success (Löfstedt, 1986). The female emitting the species specific pheromone blend will be most attractive for the majority of males and the males responding to the common pheromone has the possibility to mate with most females (Löfstedt, 1986). If there is risk for hybridization, additional separation can evolve e.g. the pheromone component from one species act as behavioral antagonists to other species (Löfstedt et al., 1991). Pheromone blends can be the primary barrier for gene flow and separate populations in sympatry (Linn and Roelofs, 1989;

Löfstedt, 1993) or the pheromone can be of secondary importance and isolate populations that already are diverged in allopatry (Löfstedt, 1993).

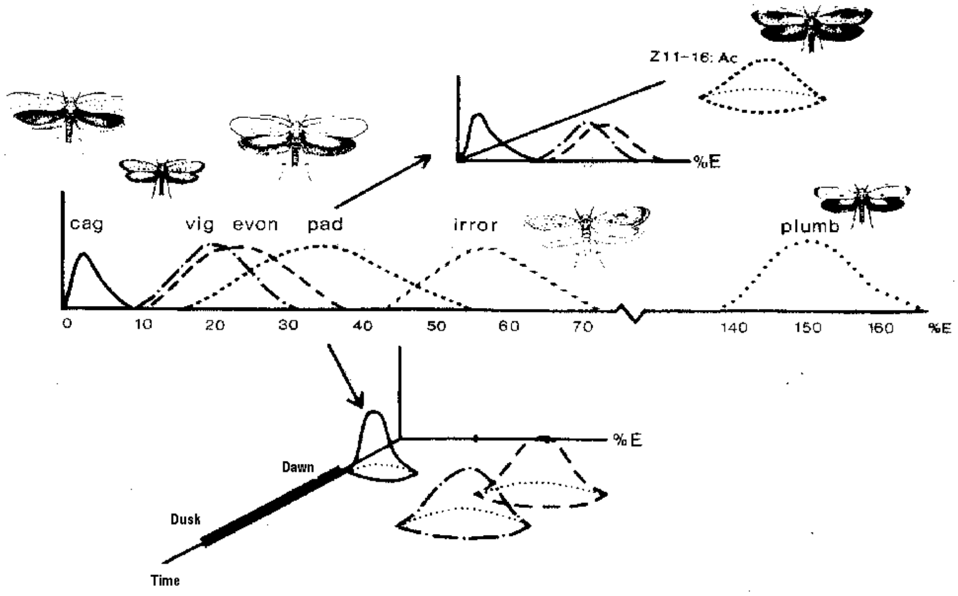


Figure 6. Graphic model of niche separation in the small ermine moth. The pheromone contains a mixture of two acetates (Z11 and E11-14:OAc), however the ratio is not species specific. If there is overlap along the Z/E-axis additional separation occurs e.g. temporal or spatial (Löfstedt, 1986).

6.2 Drosophila and olfaction

Drosophila is a model insect when speciation is studied. The data from *Drosophila* are unique – and are likely to remain so – because of the large number of crossable species and the ease of creating sexual and postzygotic isolation in the laboratory (Coyne and Orr, 1997; Coyne and Orr, 1989; Dodd, 1989).

D. melanogaster has been used to study how speciation affects the olfactory system (e.g. Dekker et al., 2006; Mcbride and Arguello, 2007; Rkha et al., 1991; Stensmyr, 2004). The *D. melanogaster* group contains closely related species occupying widely different niches. In addition, the species also display varying food preferences, with species ranging from single host specialists to true generalists (Hoy, 2003). Surprisingly, the olfactory system has to a large extent stayed unchanged over evolutionary time (Stensmyr, 2004).

Compared to the pheromone system – which for each insect only includes a few compounds – the number of volatiles emitted from fruit and plants is much higher, e.g. 230 different from banana (Macku and Jennings, 1987). Still, the insect's plant odor-detecting olfactory receptor neurons (ORNs) can match pheromone ORNs with respect to selectivity and sensitivity (Hansson et al., 1999; Larsson et al., 2001; Stensmyr et al., 2001).

Stensmyr et al. (2003) demonstrated that *D. melanogaster* only needs a few key components to locate and detect food. *D. melanogaster* is primarily feeding on rotting fruit; hence the key components are general fruit volatiles (e.g. ethyl hexanoate) as well as acetoin which indicate microbial activity. Additionally, *D. melanogaster* detects key volatiles that indicate an unsuitable resource that for drosophila are green leaf volatiles like 1-hexanol that signal unripe fruit (Stensmyr et al., 2003).

A few species exist where changes in the olfactory system has occurred, e.g. *D. sechellia* which has the *Morinda citrifolia* fruit as its only host plant. The morinda fruit has a high acid content and is toxic to all *Drosphila* species except for *D. sechellia* (Rkha et al., 1991). *D. sechellia* has adapted by loss of the olfactory receptors that respond to common fruit volatiles (ab2) in return for more receptors (ab3) that respond to the esters emitted from the Morinda fruit (Stensmyr et al., 2003).

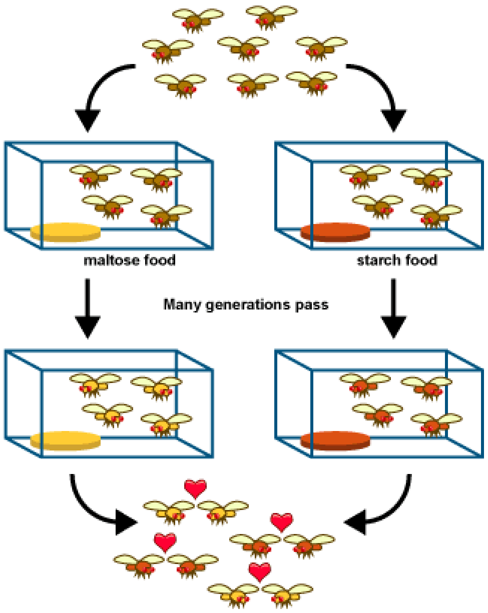


Figure 7. Stress full conditions and to populations adapting in allpatri can result in two separate species. (http://evolution.berkeley.edu/evosite/evohome.html)

Such a specialization can be the adaption to changes in the environment. Dodd (1984; 1989) demonstrated that one population can be forced to separate into two, by rearing them under stressful conditions. She divided a population of *D. pseudoobscura* into two groups that were reared on either a starch-based medium or a maltose-based medium (Dodd, 1984; Dodd, 1989). Both media where stressful and it took the populations several months to become established (Dodd, 1984). After adaption, individuals from the different groups could no longer reproduce as they were isolated by behavioral barriers (Figure 7). Hence, the selection for individuals adapted to the food had also affected the genes involved in reproductive behavior (Dodd, 1989).

6.3 Rhagoletis and sympatric speciation

6.3.1 The sibling species complex

The *Rhagoletis pomonella* Wash (Diptera: Tephritidae) sibling species complex is a model system for sympatric host race formation and speciation, and often cited as an example of host race formation in action (Forbes et al., 2005; Linn et al., 2003; Linn et al., 2005b). *R. pomonella* recently shifted from its ancestral host hawthorn (*Crataegus spp*) to domesticated apple (*Malus pumila*) which was introduced to the eastern United States in the mid-1800s (Figure 8).

Figure 8. Rhagoletis and its two hosts in the eastern United States: apple (left) and hawthorn (right). (http://www.sciencecases.org/maggot_fly/images/emergence.gif).

A third *Rhagoletis* fly that infests flowering dogwood, *Cornus florida* L (*Cornaceae*) has been hypothesized to be the sister taxon to *R. pomonella*. The hawthorn population is presumed to be ancestral, thus the dogwood and apple populations have evolved their preferences independently (Berlocher, 1999; Berlocher, 2000).

6.3.2 Tuned to the natal fruit

Rhagoletis use volatile compounds emitted from the surface of fruit to recognize their host plants (Table 4) (Nojima et al., 2003a; Nojima et al., 2003b; Zhang et al., 1999). The majority of individuals from a given host population has a strong preference for their natal fruit compared to non-natal fruit (Linn et al., 2003; Linn et al., 2005; Linn et al., 2004) e.g. the derived apple fly race has evolved an increased preference for apple fruit volatiles and decreased the response to hawthorn volatiles (Linn et al., 2003). However, within each population there is a proportion (10%-30%) of broad responders that are attracted both to their natal blend and one or more non-host blends. This variability in host discrimination is possibly the basis for the sympatric host race formation in *Rhagoletis* (Linn et al., 2005b). Host choice is of evolutionary significance for *Rhagoletis* as they mate on or near the fruit of their respective host plant (Feder and Filchak, 1999; Linn et al., 2003). Adult flies tend to mate on or near the same species of host fruit as the one they infested as larvae. Thus, differences in host preferences can translate into mate choice and can act as pre-mating barriers to gene flow (Feder, 1994, 1998, Forbes et al., 2006).

Table 4. Key volatiles attracting *Rhagoletis pomonella* from Dogwood, Hawthorn and Apple (Nojima et al., 2003a; Nojima et al., 2003b; Zhang et al., 1999).

Key volatiles from:	**Flowering dogwood**	**Hawthorn**	**Apple**
Chemical	**% in blend**	**% in blend**	**% in blend**
1-Octen-3-ol	9.1		
3-Methylbutan-1-ol	27.5	4.0	
4,8-dimethyl-1,3(E),7-nonatriene		0.07	
□-Caryophyllene	5.8		
Betyl hexanoate		0.01	37
Butyl butanoate			10
Dihydro-□-ionone		0.10	
Dimethyl trisulfide	1.9		
Ethyl acetate	54.9	94.3	
Hexyl butanoate			44
Isoamyl acetate	0.9	1.5	
Pentyl hexanoate			5
Propyl hexanoate			4
***Rhagoletis pomonella* origin from:**	**Dogwood**	**Hawthorn**	**Apple**

The attraction to the host blend is antagonized when certain non-host volatiles are added to the host blend (Linn et al., 2005a). Especially, the attraction of the apple flies to the apple blend can be antagonized by adding key volatiles from hawthorn and dogwood (Figure 9) (Dambroski et al., 2005; Linn et al., 2005a).

These findings indicate that the properties of fruit volatiles are similar to the properties of pheromones. The male sensory system is highly tuned to the conspecific female – her pheromone components are highly attractive while similar pheromone components from other species are repellent. In the same way, attractive volatiles from the host plant and the repellent volatiles from the non-host plant might increase the ability of *Rhagoletis* to find its host plant and decrease the time spent searching for a host.

Surprisingly, single sensillum recordings revealed that all fly races can detect the same volatiles (Olsson et al., 2006a). However, there is variability in the sensitivity and temporal firing of the ORNs (Olsson et al., 2006c). The ORNs (both within and between populations) have a breadth of variation in sensitivity to all volatiles (Olsson et al., 2006c). The variability in peripheral sensitivity might influence host preferences and contribute to host fidelity, but can also be the basis for a sympatric host shift. In ancestral populations the variability would provide a greater array from which changes in response to blends could emerge and facilitate the acceptance and subsequent colonization of a new host.

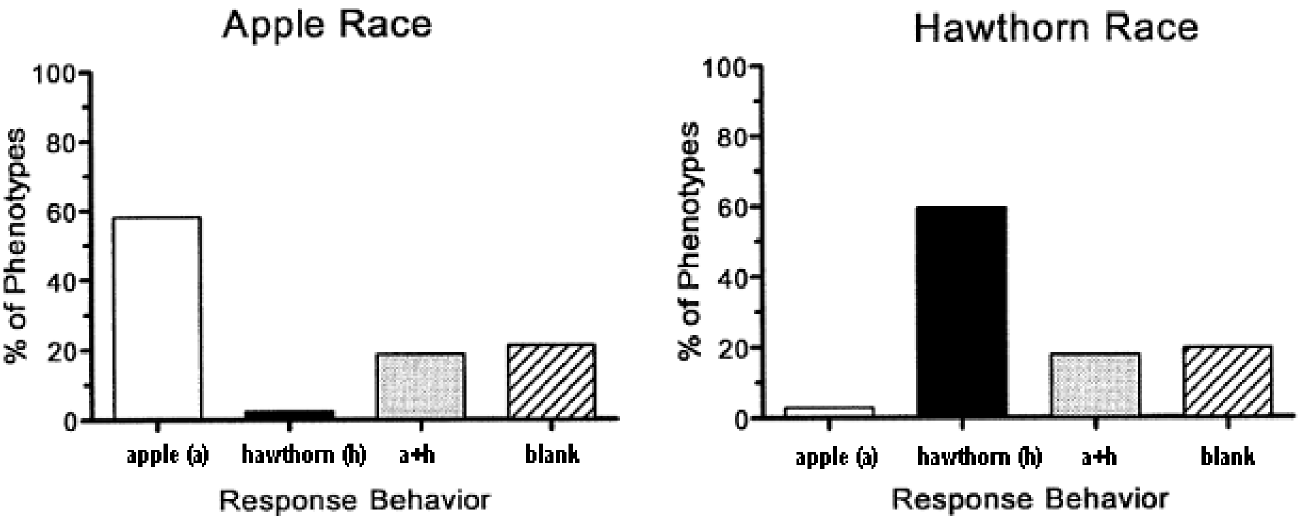


Figure 9. Most individuals from a given host population have a strong preference for their natal fruit blend compared to non-natal volatiles. However, the attraction is antagonized when certain non-host volatiles is added to the host blend (Adapted from Dambroski et al., 2005).

6.3.4 Inheritance of host preferences

Both single-sensillum recordings (Olsson et al., 2006b) and behavioral experiments (Dambroski et al., 2005; Linn et al., 2004) show that F_1 hybrid flies responded differently to host plant volatiles compared to the parent populations. This indicates that fruit odor discrimination has a genetic basis (Dambroski et al., 2005; Linn et al., 2004). Since a part of the F_2 hybrids mirrored the response of the parental flies, host discrimination is regulated by only a few genes (Dambroski et al., 2005).

The altered response of the hybrids might be caused by an alteration in the expression of the olfactory receptor neurons (Baker et al., 2006; Olsson et al., 2006b). The axon from the olfactory neuron targets the glomeruli in the antennal lobe (Anton and Homberg, 1999; Bargmann, 2006; Vosshall et al., 2000). The projection is specific; receptors that express the same receptor protein convert on the same glomerulus independently of how the receptors are distributed on the antenna (Anton and Homberg, 1999; Bargmann, 2006). This construction entails that a simple change in receptor expression can lead to a shift in perception of odors i.e. if they are attractive or repellent.

In nature, the hybrids have a reduced ability to detect and orient to host fruit (Dambroski et al., 2005; Linn et al., 2004). This is a fitness disadvantage compared to non-hybrids, as they can not locate a suitable host and – as mating is linked to the host – a suitable mate. This might contribute to the reproductive isolation of new races in the complex, as host specific mating might serve both as a postzygotic and a premating barrier.

6.4 The gall midges

The family Cecidomyiidae (Diptera), the gall midges, contains more than 5000 described species (Gagné, 2004). They form galls on almost all plant parts, and are widely distributed among host plants (Gagné, 1989). They are of evolutionary interest because of their rapid rate of speciation compared to related families of Diptera (Foster et al., 1991). At the generic level, the gall midges are often polyphagous – especially in the large genera as *Asphondylia* and *Contarinia*. However, at species level many gall midges are monophagous or oligophagous (Yukawa et al., 2005). Gall midges are unusual among phytophagous insects as closely related species can be associated with different parts of a single host-plant (Gagné, 1989; Joy and Crespi, 2007).

6.4.1 Life-history strategies

The life span of adult midges can be as short as 1-2 h, but is commonly 1-2 days (Harris and Foster, 1999). Within this limited time, the midges have to locate a mating partner, mate and the females have to locate a suitable oviposition place.

Gall midges have different life-history strategies depending on where and how they overwinter (Tokuda and Yukawa, 2007; Yukawa, 2000). Type IA and IB species overwinters as full grown larvae in the ground, without or with the gall respectively. Type IIA and IIB overwinters in the galls attached to the plant, either as mature or as immature larvae.

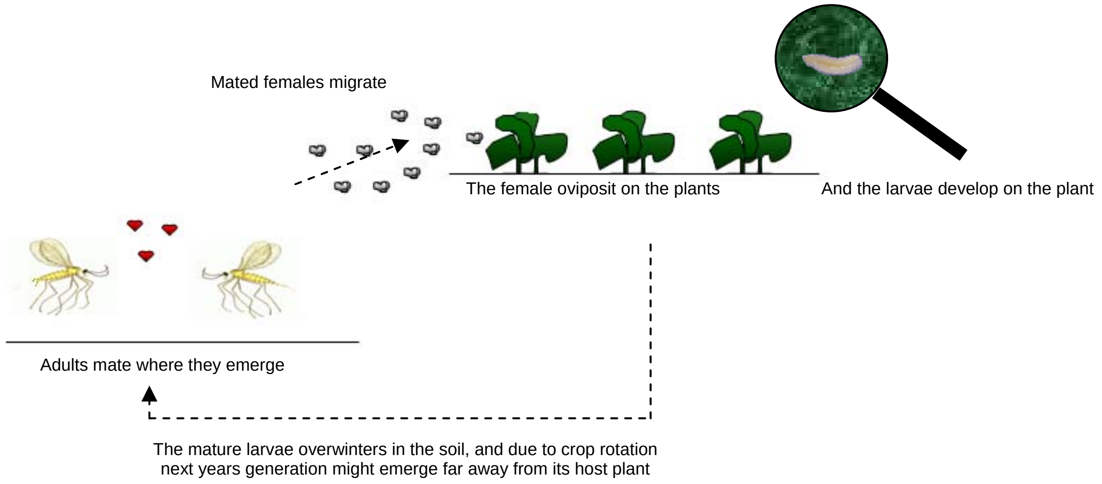


Figure 10. General life cycles for type I gall midges

6.4.2 Gall midge speciation

Speciation of plant-feeding insects is typically associated with host-plant shift followed by divergent selection and adaption to the conditions associated with the new plant (Berlocher, 2000; Funk et al., 2002; Groman and Pellmyr, 2000). However, there are examples of gall midges that speciate without a host plant shift (Joy and Crespi, 2007; Yukawa et al., 2005). Instead, speciation occurs when the gall midges associate with new parts of the host-plant e.g. move from leaf to stem (Joy and Crespi, 2007; Yukawa et al., 2005). The shift to a new plant part does not require as extensive evolutionary changes as the stressful shift to a new host plant (Joy and Crespi, 2007). Instead, within-host speciation might be facilitated by change in diapause timing and less intraspecific competition for oviposition sites (Joy and Crespi, 2007).

Within-host speciation occurs in type II gall midges. Type II gall midges are closely associated with the host plant in all stage of their live cycle, and several generation occurs in the same place. In contrast, type I gall midges only live part of their life cycles on the host plant (Figure 10) and do not display host specific mating (Readshaw, 1965; Summers, 1975; Thygesen, 1966). After overwintering and mating at the emergence site, mated females migrate to the oviposition site on the host plant (Readshaw, 1965; Summers, 1975; Thygesen, 1966). For gall midges associated with agriculture, the female often have to migrate relatively long distances (due to crop rotation) and is then guided by olfactory cues (Birkett et al., 2004; Galanihe and Harris, 1997; Pettersson, 1976). This is a crucial step in the gall midge lifecycle. The ability of the female to locate a suitable place to oviposit determines the survival chances of the offspring. Despite the importance of the oviposition choice, gall midges sometimes lay eggs on unfamiliar host plant or host plant parts (Larsson and Ekbom, 1995; Larsson and Strong, 1992; Yukawa et al., 2008; Åhman, 1981). The survival chance of the larvae is low on the alternative hosts but not always zero (Yukawa et al., 2008). The development time of the larvae depends on the properties of the host plant and can vary on different plants (Linkosalo, 2000; Mahoro, 2002). That can result in temporal isolation of the emerging adults – a possible first step in sympatric speciation (Feder and Filchak, 1999). Thus, a possible mechanism for speciation in type II gall midges is a combination of oviposition mistakes and variation in the developmental time on different plants.

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