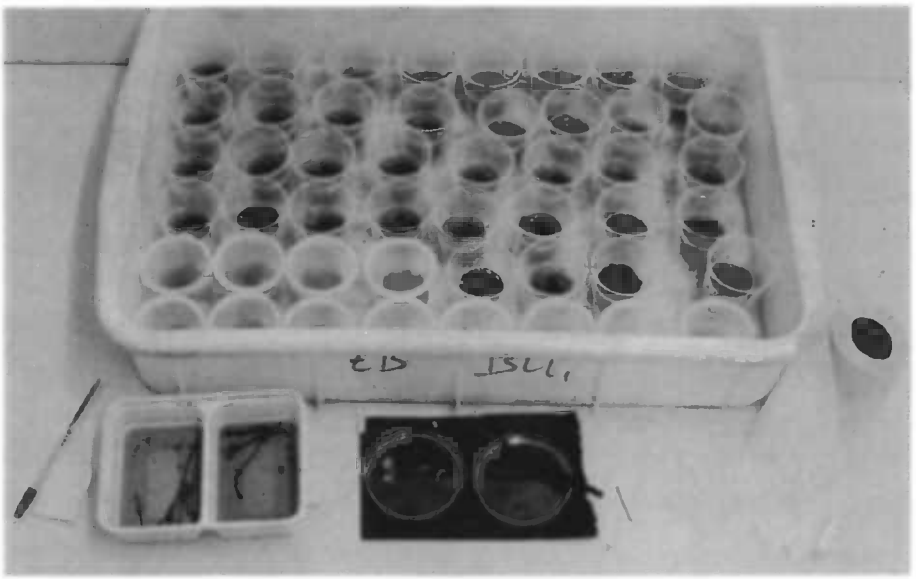
PAEDOGENESIS in *ERISTALIS ARBUSTORUM*



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M. Sc. Thesis
1999

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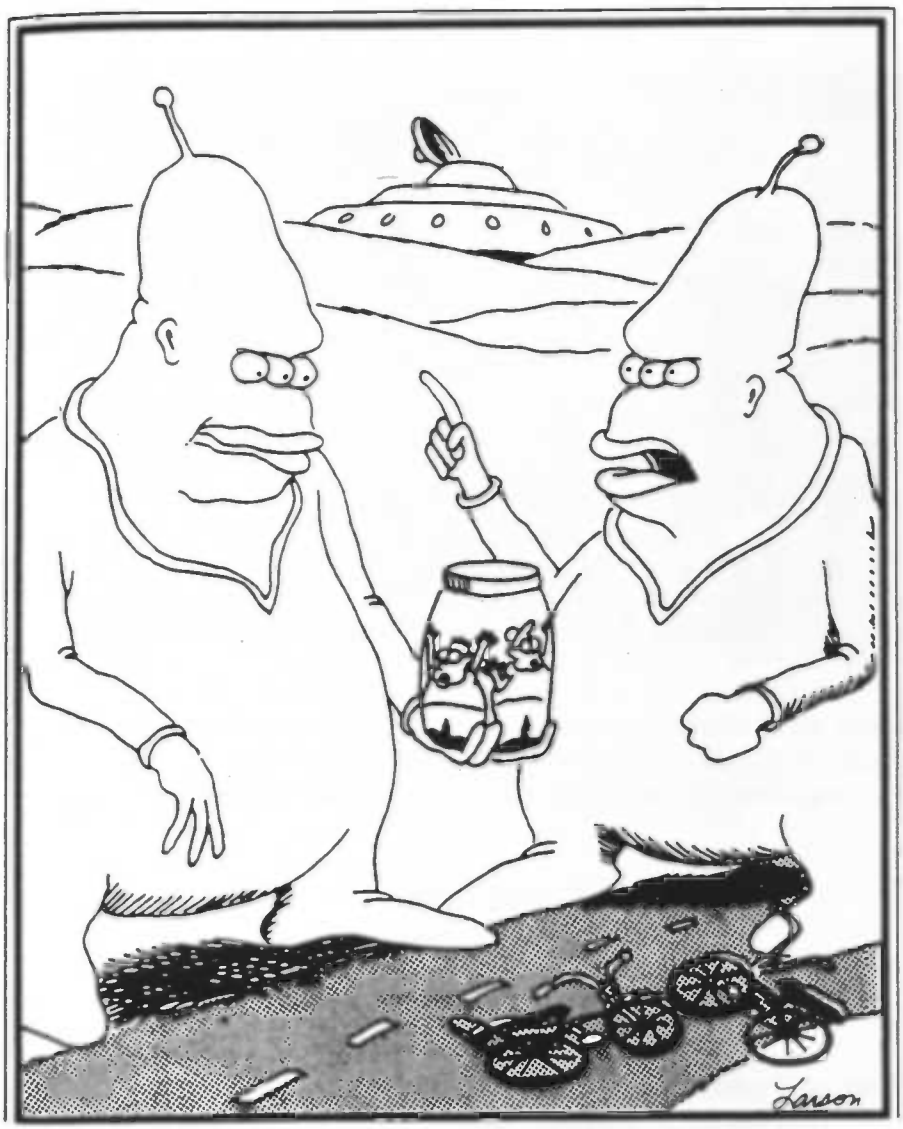
Summary
Paedogenesis is the reproduction by larvae or juveniles. In insects this form of reproduction is known from one beetle, several species of gall midges and possibly *Eristalis* hoverflies. This study aims to show paedogenesis for *E. arbustorum* under controlled conditions. The first two experiments were unsuccessful. In the third experiment, a total of 1266 larvae were reared and five occasions of paedogenesis were recorded among 542 successful pupations. In all cases of paedogenesis, one larva was put in the container and two larvae or pupae were collected later. The life history consequences of this way of reproduction are discussed.



"Now don't forget, Gorok! . . . THIS time punch some holes in the lid!"

1 Introduction

The many different animals in our world have very diverse modes of reproduction. The challenge for evolutionary biologists is to explain how this diversity has arisen (Roff, 1992). Paedogenesis is reproduction by juvenile animals*. At first sight, this is a rather strange way of reproducing because generally, the juvenile stage serves for growth, and it is only the adult's task to reproduce. Although paedogenesis is indeed relatively rare, quite a number of animals belonging to very different taxonomic groups exhibit this reproductive mode. This indicates that the adaptive value of paedogenesis lies in (probably different) ecological traits. Paedogenesis is more interesting when the difference between adult and juvenile is more profound, because then it involves more profound changes in ontogeny. Therefore the paedogenetic cases in the insect order of the Diptera are among the most interesting stories of reproduction.

In *Eristalis* hoverflies, paedogenesis would mean a sub-route to the life cycle: next to the normal sequence adult > egg > larva > pupa > adult, there is the additional route adult > egg > small larva > big larva > small larva etc.
In *Eristalis tenax* paedogenesis has been reported and photographed (Ibrahim & Gad, 1975). Supernumerary larvae were incidentally observed by Ottenheim (unpublished data) in *E. arbustorum*. The purpose of the present study is to demonstrate paedogenesis, if possible unequivocally, in *E. arbustorum* under controlled laboratory conditions.

To compare the apparent paedogenesis in *Eristalis arbustorum*, the known cases of paedogenesis in animals will be reviewed, with the emphasis on the cases in insects. It appears that in the literature, much confusion exists about the definition of paedogenesis and about the border-cases involving the related terms polyembryony, neoteny and progenesis. Understanding the terminology is important for an understanding of what is really happening in paedogenetic animals.

*) Paedogenesis (or pedogenesis) is also a geological term, meaning the process of soil formation; and yet another use is in "spore production in immature fungi". (Henderson, 1995)

1.1 Life history

Life history is the distribution of major events over the lifetime of individuals (Roff, 1992; Stearns, 1992; Daan and Tinbergen, 1997). An important life-history feature is the timing of reproduction. Insects vary widely in the timing of reproduction; in many species the reproductive organs take days to mature, whereas in other species copulation and oviposition occur almost immediately after the last molt (e.g. Boggs, 1997). But what if the optimal timing of reproduction is even earlier, before adulthood? Some species actually take the trend further and engage in paedogenesis.

1.2 Terminology

Much confusion exists about the definitions of paedogenesis and the related terms polyembryony, progenesis and neoteny (e.g. Fox and Fox, 1966). To start with, short definitions are now given from Henderson's Dictionary of Biological Terms (1995):

Term	short definition
Paedogenesis	reproduction in young or larval stages
Polyembryony	monozygotic twinning
Progenesis	maturation of gametes before the completion of body growth
Neoteny	retention of larval characters beyond normal period

Paedogenesis is reproduction by juveniles or larvae. One of the problems is that juveniles of different taxa, like adult animals, have different modes of reproduction. Reproduction by clonal budding or fission in juveniles occurs in some groups of sea-dwelling invertebrates (Bosch *et al.* 1989; Jaeckle, 1994; Craig *et al.*, 1997). In insects however, the reproduction always starts with cells from the germ-line.

Only primary reproductive organs (i.e. ovaries) need be present. Therefore, in most cases of this phenomenon there is no copulation and no fertilisation and the reproduction involves parthenogenesis i.e. 'An egg cell developing into a new individual without fertilization.' (Von Siebold, 1856; Suomalainen *et al.*, 1987). Parthenogenesis is, however, not necessary for paedogenesis, as is evident in the bat-bug *Hesperoctenes fumarius* (Hagan, 1931).

Polyembryony is the splitting of one embryo into many before the end of embryogenesis (Hardy, 1995). In humans, polyembryony may result in identical twins, and can be regarded as a 'mistake', but in some insect species polyembryony has been developed into an alternative mode of reproduction as, for example, in the parasitoid wasp *Copidosoma floridanum* (Craig *et al.*, 1995; Hardy, 1995a; 1995b; Strand and Grbic, 1997). The female lays a single egg in the egg of the host insect. While the host larva grows, the single parasitoid embryo develops into 1500-2000 morulae that subsequently follow the normal development to the larval stage.
Paedogenesis occurs after the end of embryogenesis, in the juvenile stage, when the cells are differentiated in tissues and organs. In their lengthy review of polyembryony Craig *et al.* (1997) proposed to extend the term polyembryony to "cases of pre-adult cloning". But it really does make sense to distinguish between reproduction by embryos or by larvae/juveniles and I propose to call the latter paedogenesis. In polyembryony sensu stricto it is impossible to pick out the original individual: it has split up before much differentiation has occurred. On the other hand, immediately after paedogenesis it is always evident which individual was the original one and which are its offspring, whether they develop from eggs or by fission. Maybe it can be argued that there is a gradual development from embryo to juvenile, but border cases have not been found yet (Craig *et al.*, 1997).

The maturation of gametes before the completion of body growth is termed progenesis (Giard, 1887 in Gould, 1977, p 226). In insects, copulation occurring almost immediately after the last moult is a regular phenomenon and it is therefore not surprising that mature eggs and sperm can be found in juvenile individuals. Generally fertilisation cannot take place in juveniles and reproduction is restricted to adults. Only in parthenogenetic animals is the phenomenon functionally interesting: for example, almost all aphids (Heteroptera: Aphididae) show progenesis (Suomalainen *et al.*, 1987). "Viewed from the outside an aphid first grows and then switches to reproduction. However, at birth

aphids already have embryos developing in their gonads and the most advanced embryos have also started to ovulate and develop gonads, i.e. at birth an aphid has some of her granddaughters developing within her" (Kindlmann and Dixon, 1989). But despite the early onset of their development, young aphids are only incidentally *born* before the mother is an adult. Paedogenesis is a term with an outside viewpoint, and covers cases where the egg is actually laid by or the young actually born from a pre-adult stage. Hence paedogenesis is rare in aphids, but it does occur, at least in *Schizaphis graminum* (Wanjama and Holliday, 1987). The progenesis of the rare bat-bug *Hesperoctenes fumarius* (Heteroptera: Polyctenidae) is similar, but here a peculiar, haemocoelic insemination occurs and the reproduction is sexual. Although juveniles can carry big offspring, paedogenesis resulting from this case of progenesis has not yet been observed (Hagan, 1931). Should a juvenile in this species be observed to give birth, then this would represent the first case of bisexual paedogenesis in insects. Progenesis is a prerequisite for paedogenesis in insects, but does not incorporate larval cloning in sea stars.

Sometimes one or more juvenile features occur in adults. This situation is defined as neoteny (Giard, 1887 in Gould, 1977). The adult stage and adult features can be inferred from individuals of the same species that show normal development, or from a comparison with related (even ancestral) species. The distinction with paedogenesis and progenesis becomes much clearer if one acknowledges that the difference is in the underlying mechanism rather than in its result. Neoteny arises when somatic development is retarded, whereas progenesis results from an acceleration in sexual maturation (Gould, 1977).

Gould's review of the terminology around progenesis and neoteny in his book 'Ontogeny and Phylogeny' is based on an extensive literature from Aristotle through 1976 and is the only broad overview I read*. Gould treats paedomorphosis: the retention of ancestral juvenile characters by later ontogenetic stages of descendants. This morphological phenomenon, paedomorphosis, can be the result of two distinct develeopmental processes: neoteny and progenesis. All three terms "have sensible etymologies, are faithful to their original meanings, and are widely understood" (Gould, 1977).
All very well, but one aspect of Goulds views is problematic: he restricts the term paedogenesis to the definition it had when the term was originally coined by Von Baer (1866 in Gould, see also von Baer 1864; 1865) for "parthenogenesis in insect larvae structurally unable to copulate". Subsequently it has been extended to all precocious maturation by some biologists (e.g. Carpenter, 1928; Wigglesworth 1964; Oldroyd, 1964; Chapman, 1971; Borror *et al.*, 1981; Davies, 1988), but in the entomological literature, von Baer's definition has always prevailed (e.g. Seguy, 1950; von Keller, 1963; Ibrahim and Gad, 1975; Suomalainen *et al.*, 1987; Gullan and Cranston, 1994). Therefore, Gould adopts progenesis for the broader sense of paedogenesis. He says to use Giard's definition, where the gametes form and mature before the animal has attained its complete development, but in the glossary he gives a different meaning that also shines through the whole text: "paedomorphosis produced by precocious sexual maturation of an organism still in a morphologically juvenile stage". In Giard's sense, progenesis is about precocious production of gametes in an animal that can later on develop to a normal adult, but in Gould's sense, somatic development is truncated and the adult retains characters of ancestral juveniles. Apart from this inconsistency, there are two problems with his choice:

1) Should not some significance be placed in the fact that in progenesis, the juveniles only start developing their offspring, and do not have to give birth or lay eggs to be classified as progenetic? Is the actual reproduction (although enabled by progenesis), by birth or oviposition in juveniles, not worth a separate term, paedogenesis?

2) How can reproduction by juveniles without involvement of the germ-line (e.g. larval cloning in sea stars) be classified? And what should be done with cases where the origin of the offspring, germ-line or somatic tissue, is still unknown?

These problems are solved by acknowledging the different viewpoints you take when classifying an animal as paedogenetic or progenetic. Paedogenesis is the reproduction by juveniles via whatever reproductive mode, and progenesis is the acceleration in maturation of the sexual organs relative to the somatic maturation, no matter at what stage in the life cycle of the parent the new generation is exactly produced.

How can neoteny and progenesis be distinguished in practice? Since neoteny involves a delay or retardation, the development time before reproduction should be unchanged or increased relative to related individuals. In progenesis, development time is decreased and development is often truncated (Gould, 1977). In short, progenesis and neoteny, although they can be morphologically similar, are clearly distinct. They have a different origin in ontogeny, and also very different ecological causes and consequences, as we will see later. The reproductive modes discussed in the previous paragraphs are visualised in **figure 1**.

*If I had not found these references too late in my project, I would have liked to read Wakahara, 1996; Reilly *et al.* 1997; Ryan and Semlitsch, 1998 and McKinney, 1999.

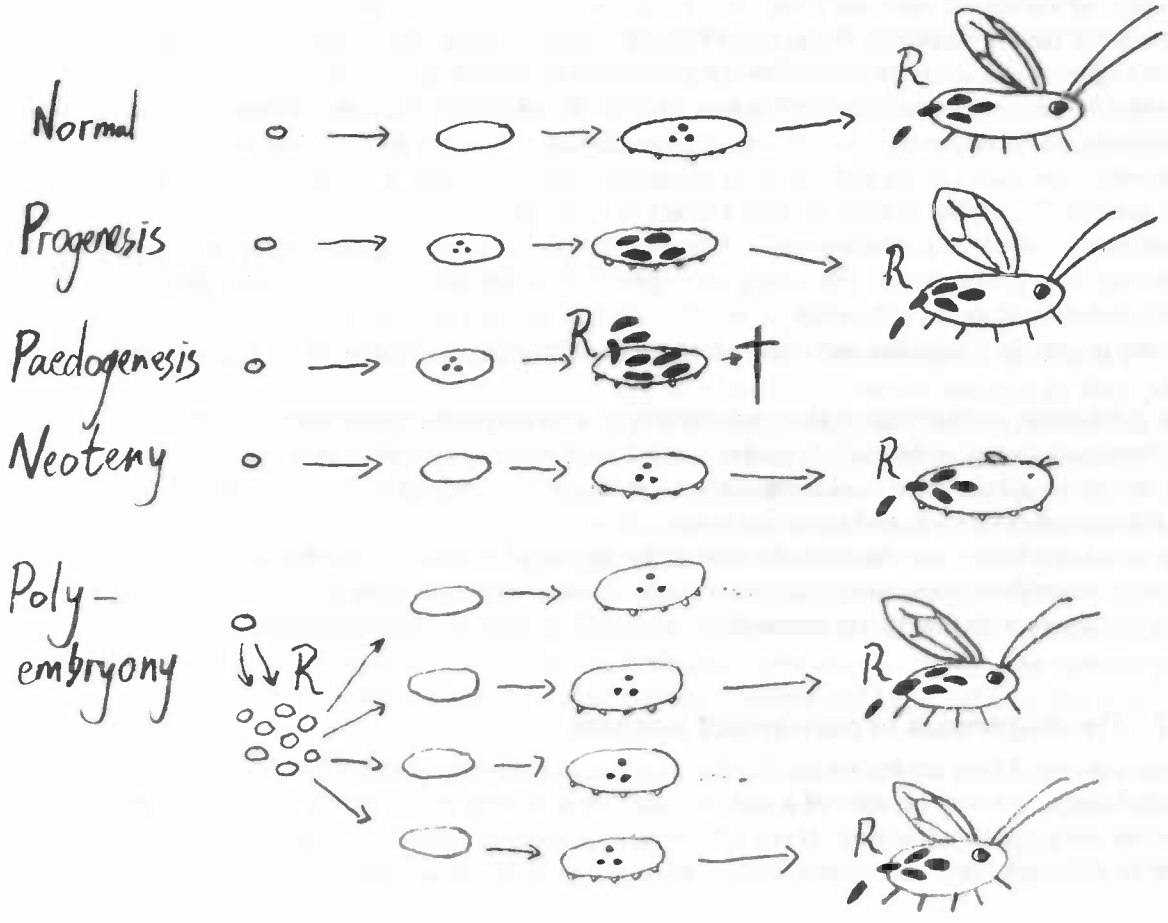


Figure 1: Different options regarding the timing of reproduction in the life cycle. Ontogeny is from left to right. Stages are an egg, a small larva, a large larva, and the adult. Start of maturation of eggs is given by three small dots and mature embryos by bigger black ovals. Actual reproduction is indicated with an R.

1.3 Bisexual or parthenogenetic reproduction?

As so many cases of paedogenesis involve parthenogenesis, a short summary of parthenogenesis in insects is useful.
If new individuals bud off the body of the old individual, this is asexual. In parthenogenesis however, new individuals do arise from sex cells, and it is in a sense not a mode of asexual reproduction. Suomalainen *et al.* (1987) adopt the definition of Von Siebold (1856): "An egg cell develops into a new individual without fertilization." The condition is derived form sexual reproduction; hence the opposite is bisexual, not sexual.
Mixis is the rearranging of genetic material through meiosis and fertilization (or in general both), and results in offspring differing from the parents. In amixis this is not the case and this term covers most cases of parthenogenesis and asexual reproduction. Unfertilized eggs sometimes develop strictly to females (thelytoky), sometimes to males only (arrhenotoky) or to both sexes (deuterotoky).

Knowledge of cytology is important in understanding the nature of parthenogenesis. From the cytological viewpoint there are three main types (Suomalainen *et al.*,1987):
Automictic parthenogenesis: Reduction of chromosome number takes place, but the diploid (zygoid, from zygote) number is before or afterwards restored by various means. The process can indeed involve meiosis and genetic recombination, but this is not always the case. Occurs in many groups.
Apomictic parthenogenesis: No chromosome reduction occurs nor fusion of nuclei. Only one maturation division occurs and this is an equational one. Apomixis is clonal reproduction, the young are, genetically, copies of their mother. Occurs in many groups.
Generative or haploid parthenogenesis: Chromosome pairing and reduction take place, but no restoring of zygoid number. The young are haploid. In all known cases, only males develop from these unfertilized eggs (arrhenotoky), whereas diploid females develop when fertilization does occur. Occurs in certain Coccoidea and Aleurodidae, a few Coleoptera, and almost all Hymenoptera.

The cytological mechanisms in the reproduction of a species might seem very rigid, and parthenogenesis hence difficult to evolve. Here I want to cite Suomalainen *et al.*, 1987: "One might say that about all chromosomal mechanisms that one might think of have been realized in parthenogenetic animals, and even that is not all."
The exact cytological mechanisms determine the degree of genetic recombination: variable in automictic parthenogens, none in apomictic, and always present in haploid parthenogenesis. The latter type co-occurs with genetic recombination in sexually produced, diploid females.

1.4 Paedogenesis in non-insect animals

Cases presented here are the result of quite an extensive literature search. Despite this, I certainly missed cases, not only because of a lack of time, but to a large extent by the fact that the phenomenon is often not properly classified . If not otherwise indicated, the cases are taken from Craig *et al.* (1997), although they were called cases of polyembryony by those authors. (see § 1.2)

1.4.1 Phylum Hydrozoa

In a sense, all hydrozoan cnidarians could be termed paedogenetic: sexual reproduction by medusae yields planula larvae which settle and form hydroid colonies which then often bud of new medusae: if hydroid colonies can be termed larvae or juveniles (which is controversial at least) , then this budding would be paedogenesis.
Cunina proboscidea (Hydrozoa: Narcomedusae), has a very complex life cycle. Large female

medusae produce eggs that can develop either sexually or parthenogenetically. The fertilised eggs develop into dwarf males. The unfertilised eggs develop into alpha-larvae that bud off beta-larvae. The alpha-larvae metamorphose into dwarf female medusae that produce eggs, which develop (without fertilisation) into large female medusae. Beta-larvae also develop into dwarf female medusae that produce eggs, which are fertilised by sperm from the dwarf male medusae. These eggs develop into large male medusae that can fertilise the eggs of the large female medusae. Only the budding of beta-larvae from alpha-larvae can be called paedogenesis.

1.4.2 Phylum Platyhelminthes

Endoparasitic flatworms: Many digenetic flukes (Trematoda: Digenea) multiply paedogenetically inside intermediate hosts. A fertilized egg yields a sporocyst, which produces many redia, who again yield many new redia. Eventually, these metamorphose into cercariae that infect the final host. It is not stated whether the offspring are produced from the germ-line.

Cestode larvae (Cestoidea: Eucestoda) also show reproduction in the intermediate hosts, e.g. Taeniid tapeworms *Echinococcus*. Within a hydatid cyst, many brood capsules are formed; within each brood capsule, many scolices bud of and the final result is up to a million proscolices per cyst (Moore, 1981).

1.4.3 Phylum Arthropoda: Rhizocephalan barnacles

The Rhizocephala are a parasitic group of barnacles (Crustacea: Cirripedia) that have such a specialised morphology and life cycle that they can only be recognised as Crustacea by their pelagic "Cyprid" larva and their sperm. The Cyprid larva finds a decapod Crustacean host, settles and soon metamorphoses into a kentrogon; this kentrogon stage injects a vermiform body into the host body from which about 25 de-differentiated cells are released that disperse via the haemolymph of the host. Then a gap in the knowledge exists, but later on the host body is infiltrated with "trophic rootlets", starting from an already differentiated stage adjacent to the ventral nerve cord. It is unclear at present if the multiplication upon kentrogon injection increases the fecundity of the "individual", because generally only one sexual externa is formed. Possibly, multiple invasions have other beneficial effects on fitness notably enabling better manipulating of the host's physiology, locating the optimal site for externa-formation or competition with unrelated interna (Glenner and Hoeg, 1995; Craig *et al.*, 1997).

1.4.4 Phylum Echinodermata

ASTEROIDEA

Larval cloning has been observed in at least two species of sea stars, *Luidia* sp. (Asteroidea: Paxillosida) and Asteroidea, a non-paxillosid species. The larvae have only been collected in plankton tows, and because adults were not obtained the species remain unknown. The life cycle in these larvae is: egg -> embryo -> primary larva-> secondary embryo-> secondary larva->adult). In both species larvae reproduce by fission: the preoral lobe or tips of the posterolateral arms can be released in various stages of development into new larvae.
One mechanism of fission occurs in both species. The tips of the arms develop into gastrulae, and the

arm becomes narrower under the tip until fission occurs and the new individual is released as late gastrula, early larva or fully formed larva.
Autotomization of the anterior portion of the preoral lobe occurs in the paxillosid larvae. The free-swimming preoral lobe develops a coelom from coelomic material of first larva, and a new gut from ectodermal cells. The original individual regenerates its preoral lobe.
The third mechanism is similar to the first, but the individual released after fission resembles an early embryo organisationally. These gastrulate and begin feeding within two days. This has only been observed in presumed paxillosid larvae.

All these modes of asexual reproduction involve a serial dedifferentiation and redifferentiation of larval tissue. In all three modes the primary developmental cycle is overlapped by a new developmental series (and becomes: egg->embryo->primary larva->secondary embryo->secondary larva). In secondary larvae the normally endodermal regions are derived from the primary larva's differentiated ectoderm (Jaeckle, 1994).

The primary ecological role of planktonic invertebrate larvae is to disperse away from parent populations and to recruit into habitats suitable for postlarval growth, development and survival. Paedogenesis increases the possibility that at least some individuals will be transported to suitable, shallow-water regions (Bosch *et al.*, 1989; Jaeckle, 1994). It probably increases planktonic existence thereby preventing the larva from settling in unsuitable deep ocean waters, and at the same time paedogenetic larvae make a sensible use of their nutrient intake. Disadvantages are probably the small part of the soma that is lost, and the reduced feeding efficiency during paedogenesis (Bosch *et al.* 1989; Jaeckle, 1994).

OPHIUROIDEA

Less certain is the occurrence of paedogenesis in an unidentified *Ophiopluteus* species (Ophiuroidea). The larva apparently releases a juvenile to the benthos, subsequently returns to water column where it regenerates its ciliated bands and the posterior digestive system and resumes feeding. It is however not known if a second adult rudiment can be produced (Mortensen, 1921, in Craig *et al.*, 1997).

1.4.5 Paedogenetic salamanders

Many salamanders have adults that resemble larvae, for example in their external gills. Most interesting in this respect are the facultative paedomorphic species in the genus *Ambystoma*, e.g. the famous Axolotl *A. mexicanum*. Paedomorphosis is often a plastic character in *Ambystoma*, and whether individuals metamorphose depends on the conditions of the breeding pond. It is however to a large extent genetically fixed. In some subpopulations, most individuals metamorphose, whereas individuals from other locations can only difficultly be induced to metamorphose in the laboratory. The phenomenon culminates in the perennibranchiate (=eternal-gilled) salamanders that die if forced (e.g. by hormone treatments) to metamorphose, or cannot be induced at all. The popular literature and the information on the internet is very confusing about whether the larva-like morphology is due to paedogenesis (progenesis) or neoteny, and it is in this salamander literature that these terms are synonymized most often. Gould discusses the paedomorphic salamanders at length and concludes that in all examples the development time to maturity is equal or delayed in paedomorphic individuals. Therefore, paedogenesis does not occur and all cases are due to neoteny (Gould, 1977). There is however a record of paedomorphism "through the process of predisplacement -an earlier onset of maturition-" in *Ambystoma talpoideum*, where it is concluded that "the age at maturation is the principal target of selection". Although it is not stated relative to what the maturation is earlier (relative to metamorphosing individuals, or to somatic development in the same individual?) this

sounds like paedogenesis (Ryan and Semlitsch, 1998). However it remains to be seen if paedogenetic salamanders exist at all.

1.5 Paedogenesis in insects

Paedogenesis has been reported in the following insect taxa: aphids (Homoptera: Aphididae), a bat-bug (Heteroptera: Polyctenidae), a beetle (Coleoptera: Micromalthidae), gall midges (Diptera: Cecidomyiidae), chironomid midges (Diptera: Chironomidae) and hoverflies (Diptera: Syrphidae) (Imms, 1934; Richards and Davies, 1977; Daly *et al.*, 1978; Chapman, 1982; and specific references)

1.5.1 Paedogenesis in Hemiptera

APHIDS

Aphids in general (Sternorrhyncha: Aphididae) (or Aphidoidea, depending on taste) are the most famous example of progenesis. A generalised life cycle involves numerous all-female parthenogenetic generations in summer, followed by one bisexual generation in autumn that results in fertilised, cold resistant eggs. This type of life cycle is called cyclical parthenogenesis. The life cycle is further complicated by different polymorphisms. For example in summer, in the parthenogenetic colonies, both winged and unwinged adults are produced. The unwinged individuals are more like juveniles in morphology than the winged ones.

It is in both these parthenogenetic all-female types that progenesis is the rule. Three generations are often said to be telescoped into each other. This means that the first embryos start development before their grandmother reaches adulthood (Kindlmann and Dixon, 1989). Despite this acceleration, young are only incidentally born before the mother is an adult and hence paedogenesis is rare. It has only been confirmed in *Schizaphis graminum* (Wanjama and Holliday, 1987).

Both winged and unwinged females of this species produce a few young before the final molt, but development is never truncated: only the first few offspring are produced by paedogenesis. All in all, paedogenesis is unimportant in aphids, and the related term progenesis is much more suitable to characterize this group.

BAT-BUG

Aphid-like paedogenesis occurs in *Hesperoctenes fumarius* Westwood (Heteroptera: Polyctenidae), a rare parasite on bats (Hagan, 1931). Embryonic development of the young starts when the mother is still a nymph. However, all offspring are born during the physical maturity of the mother. The species has haemocoelic insemination, which enables the combination of progenesis with bisexuality. Whether the progenesis sometimes leads to paedogenesis is not sure (Hagan, 1931).

SCALE INSECTS

The Scales (Sternorrhyncha: Coccoidea) have degenerate females that are often called neotenic, but intriguingly the females have only two or three immature stages, whereas the males have generally four. This suggests that the females are paedogenetic rather than neotenic. Many morphological features of scale insects are related to their specialised life style as sedentary plant parasites with an extreme sexual dimorphism. The males of scale insects are exceptional among the (hemimetabolous) Hemiptera in having a complete metamorphosis with two pupal stages. This sexual dimorphism devaluates the comparison of number of stages between males and females. The females are often

described as "sexually mature nymphs" and this is also more fitting for paedogenesis than for neoteny. Critical would be a comparison of the development time, not measured in stages but in days. In an unidentified species, resembling *Pinnaspis strachani*, male development took, on average, 22.6 days whereas mean female development time was 44.5 days (Fernandez *et al.*, 1996). If differences of this magnitude are common, the neotenic state of Coccid females is confirmed. In fact, nobody has ever called a scale species paedogenetic, although statements like the above-cited are common, often together with the designation neoteny. (Scalenet, Ben-Dov *et al.*, 1999)

1.5.2 Paedogenesis in Coleoptera

RHYZOSTYLOPS

One dead female is all that has ever been found of the genus *Rhyzostylops*, belonging to the Rhipiphoridae. She had a very strange morphology including many degenerate characters, e.g. no wings and simple mouthparts. Adjacent to her were 3000 eggs, from which triungulin larvae hatched that were unfortunately not reared further (Silvestri, 1905).
The individual has also been interpreted as being not a female, but a larva (Riek, 1955 in Svacha, 1994). However on morphological grounds it is probably really an adult female, and "otherwise we would have to postulate paedogenesis which is unknown in Rhipiphoridae" (Svacha, 1994).

MICROMALTHUS

Micromalthus debilis is the only known beetle with paedogenesis. It is the sole representative of the family Micromalthidae, belonging to the primitive Archostemata. It is widespread in eastern North America, and also occurs in South Africa where the species has supposedly been introduced (first record from 1932, Pringle, 1938). The larvae live in and feed on rotten wood. The life cycle is complex and unfortunately not entirely known; the last original work, apart from some phylogenetic investigations, is from 1941.
The paedogenetic, thelytokous female larva produces eggs, 4 to 20 depending on environment, which mature into embryos and larvae inside her body. The first instar triungulin larvae, all females, hatch from their dead mother, eat her remains and then disperse in the wood, running fast on long jointed legs. The second and third stages are leg-less. After the third larval instar, the offspring can basically follow three developmental pathways: they can pupate and become an adult female, they can moult into the paedogenetic "larva" or they can moult into a male-producing larva. Note that paedogenesis occurs not in the third stage larva, but involves an extra larval instar, or a degenerate pupa. The production of males requires even a whole extra generation.
The male-producing larva produces one big egg, which adheres to its body. From this egg, a male larva hatches, which eats his mother instead of wood. When she is completely eaten, within a week, the male pupates and emerges as an adult. If the egg fails to develop, the male-producing larva produces a small number of female thelytokous larvae. This has led some to describe this female-producer as an additional larval type. (Barber, 1913; Scott, 1938; Pringle, 1938; Suomalainen *et al.*, 1987).
The factors triggering the production of adult females or males are unknown, but in the laboratory temperature alone is sufficient. In North America, adults are most common in August.

The different types of reproductive females are all diploid (2n=20). The male develops through parthenogenesis and is haploid in soma and germ-line. The first spermatocyte division is completely abortive with regard to the division of the chromosomes and the cell. A unipolar spindle is, however, found. The chromosomes in the first anaphase resemble mitotic chromosomes. They move away from the single pole with their centromeres hindmost. The second spermatocyte division is in all respects a normal mitosis and ultimately results in two normal sperm (Suomalainen *et al.*, 1987)

The adults can fly and could represent the dispersal and genetic recombination stage in a typical case of cyclical parthenogenesis. However, adult females have only two mature eggs in their ovaries, and mating has never been observed. In some populations, females probably never carried eggs (Scott, 1938), and in the South African populations, not a single male has been reported (Pringle, 1938). Therefore it is questionable if there is a functional bisexual phase in the life cycle of *Micromalthus*. There could be an evolution towards obligate thelytoky.

1.5.3 Paedogenesis in Cecidomyiidae

SYSTEMATICS

Paedogenesis has been reported in the following species of gall midges: *Mycophila lampra* (?) (Moehn, 1960); *Brittenia fraxinicola* Edwards, *Frirenia* sp. Kieffer, *Henria psalliotae* Wyatt, *Heteropeza pygmaea* Winnertz, *Heteropezina* sp. Pritchard, *Heteropezula tenuis* Wyatt, *Leptosyna nervosa* (Winnertz), *Miastor castaneae* Wyatt, *Miastor metraloas* Meinert, *Mycophila barnesi* Edwards, *Mycophila nikoleii* Moehn, *Mycophila speyeri* (Barnes), *Nikandria* sp. Mamajev, *Tekomyia populi* Moehn (Wyatt, 1967); *Aprionus* sp. Kieffer, and *Moehnia* sp. Pritchard (Mamaev & Krivosheina, 1993). The phylogeny in gall midges in not without debate, but it is agreed that *Mycophila* and *Tekomyia* belong to the most primitive subfamily, the Lestremiinae. The other paedogenetic species belong to a different tribe and are allocated to the subfamily Cecidomyiinae (Wyatt, 1967; Roskam, 1985; Mamaev and Krivosheina, 1993). Because paedogenesis has not been recorded in Mycetophilidae and Sciaridae, the supposed predecessors of gall midges, it seems to have evolved at least twice in this group.

Paedogenesis was first discovered in Kazan, Russia in 1861 by Wagner (1862; 1863): His mass of reproducing larvae turned out to belong to *Miastor metraloas*. After initial disbelief, the phenomenon attracted considerable interest in the following years. Biologists meticulously studied the morphology, and compared the phenomenon to other cases of parthenogenesis. Kahle (1908) reviews most of this older literature, with a detailed review of the morphology of paedogenetic larvae. At least two species of paedogenetic gall midges had been found by then: *Miastor metraloas* Meinert (Kazan, Russia by Wagner; Denmark by Meinert and Leipzig, Deutschland by Kahle himself). The other species is presumably in all cases *Heteropeza pygmaea* Winnertz 1846 (under the younger synonym of *Oligarces paradoxus* Meinert 1866), and had been found in Heidelberg, Germany by Pagenstecher already in 1864; Charkow, Russia by Ganin; Giessen, Germany by Leuckart and Metschnikoff and in Denmark by Meinert.

LIFE-CYCLE

The species that has been best studied is *Heteropeza pygmaea* Winnertz. **Figure 2** depicts the life cycle. After mating the adult *H. pygmaea* female lays a very small (1-4) number of large eggs. From the eggs, female larvae hatch that feed on fungal mycelia. Very soon, eggs start to develop in their ovaries from germ line cells, and mesodermal cells form a nurse chamber. Subsequently other mesodermal cells form a follicular epithelium surrounding the oocyte-nurse chamber complex and the resultant follicles are released in the haemocoel. Growth of this mother larva, oocyte growth and embryogenesis occur at the same time, and within a week the mother larva moults into a "hemipupa" which remains hidden under the larval cuticle and her body is completely histolysed to the benefit of her progeny. The embryos have then matured into larvae and now hatch from the dead mother to start a paedogenetic life of their own. Under suitable nutritional conditions, paedogenetic reproduction can

go on for at least 250 generations (Ulrich, 1936). Under certain conditions new-born larvae follow a different development, leading to pupae and adults of both sexes. (Kahle, 1908; Ulrich, 1936; Wyatt, 1963; Suomalainen *et al.*, 1987).

The daughter larvae can, instead of producing female young, pupate and become an adult female. Because the larvae are all female, male production needs an extra generation. Male-producing larvae develop a small number of male larvae, somewhat larger than daughter larvae. These pupate to become adult males. In fact, male producing larvae can also produce daughter larvae and male larvae together and in all ratios. Development towards one of the larval types is determined by nutritional conditions, crowding, and probably also climate and season. Remarkably, rearing the larvae on one fungus triggers development from young larvae towards male-producers, whereas another species of fungus stimulates immediate pupation and adult female production. Female larvae that are already advanced in development can still change and become female- or male-producers (Harris, 1923; Ulrich, 1936; Camenzind, 1966).

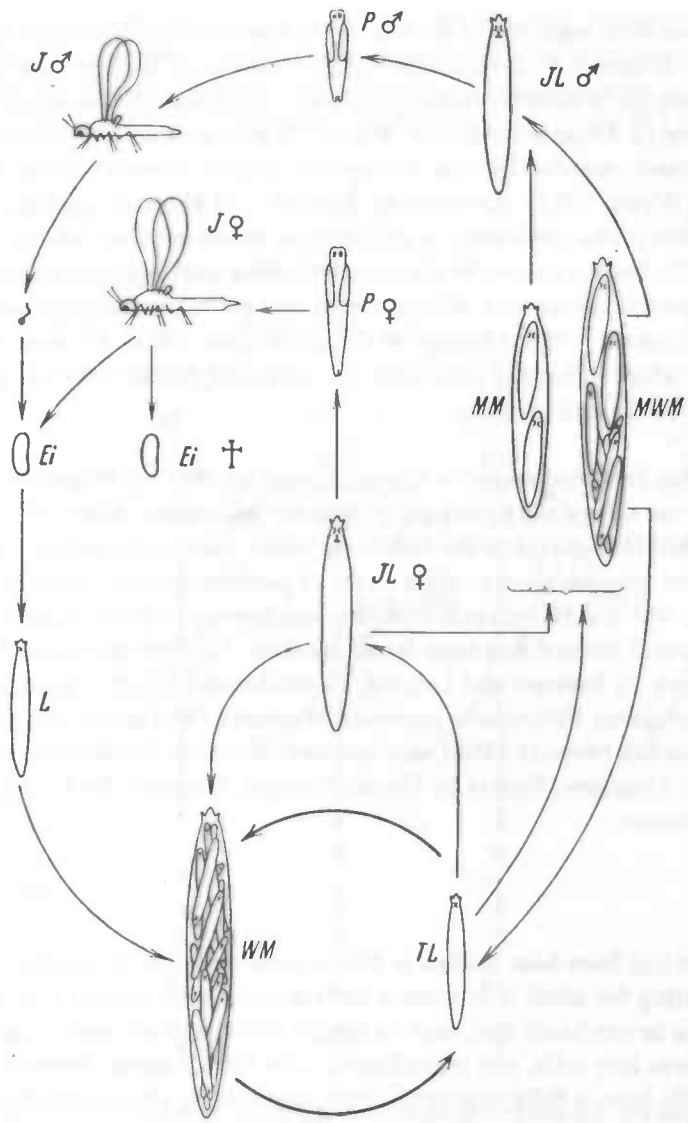


Figure 2: The scheme by Ulrich (1936), of the life cycle in *Heteropeza pygmaea*. WM= thelytokous paedogenetic larva, TL= daughter larva, JL= male and female imago larvae, MWM= deuterotokous paedogenetic larva, MM= arrhenotokous paedogenetic larva, P=pupa, Ei= egg, L= egg larva: always develops to female thelytokous larva. Unfertilised eggs died in Ulrich's strain, but are viable in other strains, and also develop always into a thelytokous paedogenetic larva.

The other species differ in number of larval stages, the number of offspring, the resemblance between hemipupa and real pupa, and the tendency to form adults. There is a correlation between these variables: the two species where the hemipupa and pupa are most similar (*Henria* and *Tekomyia*) have three larval stages in both paedogenetic and normal development, have longer development times, and produce more young per individual. Presumably there is an evolutionary trend towards shorter development times and fewer young. *Heteropeza pygmaea* has the most altered and reduced life cycle and the most degenerate adult of all known species (Wyatt, 1961; 1967).

Many authors have overlooked the fact that the stage giving birth in *Heteropeza* is a hemipupa rather than a larva, as Wyatt argues. Only Pagenstecher, the first to observe paedogenesis in this species, did remark the extra moult just before the hatching of the next generation (Pagenstecher, 1864). In fact, a hemipupal stage has been confirmed for most species; only in *Mycophila* sp., the last moult seems to be suppressed. In the Lestreminae (*Mycophila* and *Tekomyia*) the mother is alive until or even after hatching of her young (Moehn, 1960; Wyatt, 1963; 1967).

SEX DETERMINATION

This way of cyclical parthenogenesis only works with specific sex determination mechanisms, because a female larva has to be able to produce both male and female offspring parthenogenetically. Unfortunately, despite a wealth of cytological research in *Heteropeza pygmaea*, *Miastor metraloas* and *Mycophila speyeri*, this mechanism is still unknown. Females have five or ten chromosomes in their somatic tissue, whereas males have always five. Hence, male haploidy/female diploidy is ruled out as the mechanism (Went and Camenzind, 1980). The chromosomal relationships are extremely complex, but part of this complexity is common in all gall midges and the relevance for paedogenesis is unclear (Suomalainen *et al.*, 1987).

CYTOLOGICAL MECHANISMS

The cytology of the three species studied in some detail is essentially similar. In *Heteropeza pygmaea*, the germ line has 55 (Panelius, 1968 and 1971, in Suomalainen *et al.*, 1987), 66 (Reitberger 1940 (in Suomalainen *et al.*, 1987) ; Camenzind, 1966) or 77 chromosomes (Hauschtek, 1962, in Suomalainen *et al.*, 1987). The cytology is depicted in **figure 3**.

In continuous paedogenetic development, the thelytokous eggs with 66 chromosomes undergo a single equational maturation division. Therefore the parthenogenesis is of the apomictic type and the offspring are clones of the mother. At the third cleavage, 55 chromosomes are eliminated in the cells destined to become the somatic tissues. At the sixth to eighth cleavages, one more chromosome is eliminated and the somatic cells of paedogenetic females contain accordingly 10 chromosomes. Only the germ line keeps the maternal 66 chromosomes.

In the eggs of adult females there is not only the oocyte nucleus, but also two or three small nuclei with 10 chromosomes, which presumably originate from maternal somatic tissue. In the oocyte nucleus, the normal meiosis takes place and results in a reduced nucleus remains with 33 chromosomes, while the polar nuclei degenerate. The fertilized egg contains the reduced oocyte nucleus, two or three small nuclei and two or three sperm nuclei, which have 7 chromosomes. The total chromosome number in the primary cleavage nucleus of the bisexual eggs is therefore variable; typically 67 or 77 from the oocyte nucleus, two sperm nuclei and two or three small nuclei. The first chromosome elimination occurs in the third to fifth cleavage divisions. All but 6 are eliminated from the future somatic tissue. A second elimination reduces this number to 5. It is unknown at present how the number in the germ line is restored to 66.
The adult eggs of some strains of *H. pygmaea* can also develop parthenogenetically. The primary cleavage nucleus contains 53 or 63 chromosomes from the reduced oocyte nucleus and the small

nuclei. Following two eliminations, similar to those in fertilized eggs, the somatic nuclei contain 5 chromosomes. This process is automixis but note that the chromosome number is not restored by polar nuclei, but by the peculiar "small nuclei" of somatic origin.

Eggs in male-producing larvae also undergo fusion with small nuclei and subsequent reduction of chromosome number in somatic tissue. The primary cleavage nucleus is formed by the reduced egg nucleus and generally two small nuclei so that it has 53 chromosomes. The spindle formation in the first metaphase can have several forms: the small nuclei have one spindle together with the oocyte nucleus; or the small nuclei have a common spindle separate from the spindle of the egg nucleus, whereby the two spindles later fuse during late prometaphase; or they have no spindle at all, and the small nuclei migrate to the equator of the spindle formed by the egg nucleus. A first elimination results in 5 or 6 remaining chromosomes. In the latter case one additional chromosome is destroyed in a second elimination, arriving also at 5 chromosomes in the somatic tissue. The germ line keeps the number of the primary cleavage nucleus.

Spermatogenesis in adult males of all gall midges studied so far is a very peculiar process: the first meiotic spindle is unipolar. Only a few (7 in *Heteropeza*) chromosomes migrate to this pole, the others stay where they are and degenerate later. The second maturation division is a normal mitosis, and finally two sperm are produced with 7 chromosomes (Camenzind, 1966; 1971; Suomalainen *et al.*, 1987).

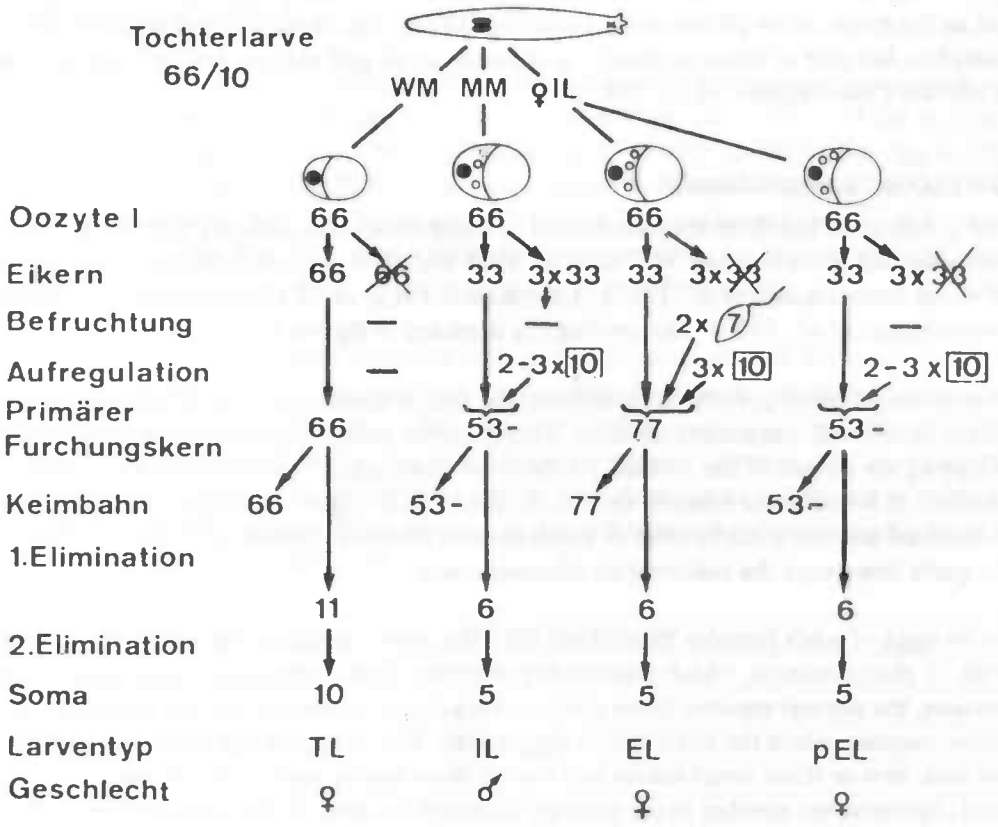


Figure 3: This picture from Camenzind, 1966, summarizes the cytology of reproduction in *Heteropeza*. The totipotent daughter larva can result in the end in four types of offspring, namely daughter larvae produced by paedogenesis (TL), male larvae produced by paedogenesis (IL), egg larvae produced in bisexual reproduction (EL), and egg larvae produced by parthenogenesis (pEL). Polar bodies from the primary oocyte always degenerate. Small nuclei are indicated by open dots. Oozyte I means primary oocyte, Eikern means egg nucleus, Befruchtung means fertilization, Aufregulation means restoring the chromosome number, Primaerer Furchungskern means primary cleavage nucleus, Keimbahn means germ-line and Geschlecht means sex. Chromosome numbers are indicated, for variation in these numbers see text.

In non-paedogenetic gall midges there seem to be two types of chromosomes, one type (E) that is eliminated from the somatic tissue and only occurs in the germ line, and the second type that remains also in the somatic cells (S). A reduced egg nucleus is said to contain a full set of E chromosomes and half of the S chromosomes, which is a haploid set. Sperm then contains the complement haploid number of S chromosomes. This could not work in *Heteropeza*, because the oocyte nucleus is reduced to half in meiosis, and sperm contain far less chromosomes (7) than needed to restore the original germ line number. The small nuclei of maternal somatic origin are needed to restore this number, and this system results, in each bisexual generation, in a net replacement of would-be germ-line chromosomes with chromosomes of somatic origin (Camenzind, 1966; Camenzind, 1971). *Mycophila* has not only small nuclei in paedogenetic male production, but also in paedogenetic female production. These small nuclei however degenerate and have no obvious function (Camenzind, 1971).

All these complex cytological phenomena do not tell very much at present. The function of the high germ-line number is unknown. One theory is that these germ-line chromosomes serve as a source of nucleoprotein molecules for the developing embryo (White, ?). Whatever function it may have, the cytology does not need to be this complex to enable cyclical parthenogenesis itself.

1.5.4 Paedogenesis in other Diptera

CHIRONOMIDAE

Paratanytarsus grimmii (Diptera: Chironomidae) is a parthenogenetic midge that starts laying eggs within two hours of eclosion. Often, the midge even fails to eclose and the adult will lay its eggs within the pupal skin. This is egg laying by the pharate adult, but it was sometimes erroneously referred to as paedogenesis (e.g. Wigglesworth, 1938). The egg-string swells as it absorbs water and ruptures the pupal abdomen, allowing the larvae to escape when they hatch. The capacity to lay eggs without eclosion has enabled P. *grimmii* to colonise water distribution systems, and its frequent occurrence in aquaria may be due to its arrival in tap water. No males are known in its world-wide range. (Langton and Cranston, 1988)
This reproduction by a pharate adult is possibly only the first step towards paedogenesis, and it would be interesting to look at the timing of development of the ovaries in *Paratanytarsus*. The nomenclature of the species is confusing; see Langton and Cranston, 1988.

There is another record related to paedogenesis in Chironomidae, a record with a high degree of obscurity. On the shore of St. Anne's-on-Sea in England, a full-grown larva determined as *Chironomus dorsalis* was caught and put individually in a jar to allow complete development. "It sickened and died, but from the decaying body came forth a large number of young *C. dorsalis*." But in the next sentences, the author Swainson gives three differences between these larvae and normal *C. dorsalis*, regarding both morphology and behaviour (Swainson, pers. comm., 1892, in Theobald, 1892). Therefore, the story is difficult to believe.

1.5.5 Paedogenesis in *Eristalis*

ERISTALIS TENAX (DIPTERA: SYRPHIDAE)

Last but not least, paedogenesis has been reported and photographed in *Eristalis tenax* (Ibrahim and Gad, 1975).
They observed three instances of paedogenesis. In one case two larvae were seen crawling from one large larvae, and alarmed by this event, Ibrahim and Gad watched the larvae for similar cases. The next case was photographed and the picture published. The photograph shows two larvae that are leaving their mother through a slit near the anus, while a third is still inside. Subsequently eight trays were set up with five larvae each; after a few days, four big larvae and three small ones inside the fifth were seen in one of the trays. Because the circumstances in which paedogenesis occurred are only briefly described, I asked Mr Ibrahim and Mr Gad a few questions in a letter I sent (see appendix at back cover) to them and also to a number of recent co-authors, but unfortunately I have had no response as yet.

ERISTALIS ARBUSTORUM (DIPTERA: SYRPHIDAE)

During a study on phenotypic plasticity in the *Eristalis arbustorum*, Ottenheim (unpublished data) accumulated a few unexplained cases with supernumerary larvae. In a few cases he knew the exact number of larvae that were put in a tray, and the number of prepupae that were crawling from it to pupate. These are depicted in **table 1**.

Results in summer 1993: From the egg-laying medium ten larvae, that had apparently escaped attention during the egg stage, were collected and put in a normal tray with 100 ml of the normal medium, rabbit dropping soup with 20 g/l yeast (Ottenheim and Holloway, 1995). The egg-laying medium consisted of water with rotting *Senecio jacobaea* leaves and must have been rich in alkaloids, because these are water-soluble. The ten larvae were 5 to 7 mm long excluding the tail and were probably in their second instar. From this tray sixteen larvae pupated in two cohorts of eight animals. The cohorts differed in development time by only twelve to twenty-four hours.

Results in summer 1994: Two trays with dropping soup yielded supernumerary larvae. A check on the above observations was performed, by putting a total of fifty-four larvae of various size, most probably second instar, in various numbers (one to six) in seventeen trays, with the same medium. The larvae within each tray were of similar size. They were collected in diverse sites in the egg-laying media with *Senecio*. Again, one larva split into two larvae. This time it was not known whether the timing of pupation was different or not. What is clear, is that it happened with a relatively large larva with a length of at least 10mm excluding tail.

Initial number	Number of pupae
10	16
20	28
20	23
1	2

Table 1 Observations by Ottenheim (unpubl.) of four cases of possible paedogenesis in *Eristalis arbustorum*. The initial number refers to the small larvae in one tray. Ottenheim reared those larvae, except the last one, for other purposes. The number of prepupa that left the medium to pupate was monitored.

The purpose of the present study was to demonstrate paedogenesis in *E. arbustorum* under controlled laboratory conditions.

2 Materials en Methods

2.1 Eristalinae

The species of this subfamily of the Syrphidae (flowerflies or hoverflies) are moderately large Diptera. They have aquatic filter-feeding larvae with a long breathing tube (the so-called rat-tailed maggots), which live in diverse semi-aquatic habitats, although not in open water. A small number of species is characteristic for very rich conditions, e.g. wet dung (Syrph-the-Net datafiles, Speight *et al.* 1999). These are also the species with potentially the shortest development time: one of the reasons to choose *E. tenax* and *E. arbustorum* for our study. Both species are very common in The Netherlands.

2.2 General rearing procedures

Because *Eristalis* larvae are extremely difficult to find in the field and can not be determined to species level unambiguously (Dixon, 1960; Hartley, 1961), the very best way of getting the larvae is from eggs laid by gravid females that are collected in the field. Gravid females were collected in and around Leiden, Breda and Zwolle (The Netherlands), in May and again in August 1999. These flies were kept in gauze cages with a honey-pollen solution and flowers, mainly *Senecio jacobaea*, *Valeriana officinalis*, *Angelica sylvestris* and various yellow crucifers. Apart from providing food, these flowers have a second very important function; they prevent the animals from flying against the gauze again and again, which would destroy their wings. A tray was placed in the cages with rotting flower stems and some water. Females tended to land on the flower stems and laid up to 150 eggs just above the water surface. Flowers were put in fresh water every day. Every three days, the flowers and the artificial food were replaced. The cages were situated indoors behind a window facing south and received direct sunlight. The temperature in the room was between 20 and 25 ^{o}C most of the time.

2.3 Egg batches

New egg batches were collected daily and placed on fresh water to which a few pieces of *Senecio* leaf were added. The egg batches of *E. arbustorum* form a very loose structure and fall easily apart upon touch. Sometimes, a few eggs that had fallen onto the water surface were arbitrarily assigned to a batch. In a few instances, two or more batches were laid so close to each other that the borderline was unclear and they had to be considered as one batch. The petri dishes were put in a climate room with a temperature of 20^{o}C. Fresh or unfertilized eggs are bright white; second day developing eggs turn greyish with a darker patch at both ends. Hatching generally took place after two days, but dependent on temperature in the egg-laying cages sometimes after one day, or only after three days.

2.4 Rearing media

The two kinds of media that were used in this study are described here roughly. Details differ and these can be found in the descriptions of the three rearing bouts. The principal idea was that paedogenesis would be stimulated by a transition from a bad habitat to a good habitat. The experiments were performed at 20°C and a 16:8 L:D light regime.

The optimal medium was made according to Ottenheim and Holloway, 1995 and consisted of rabbit dropping soup with 20 g/l yeast. The rabbit droppings were gathered in the coastal dunes near Katwijk, in the vicinity of Leiden, The Netherlands. Wild rabbits occur in high density in this nature reserve and their droppings can be easily collected in the short, sparse vegetation. The droppings were put on a sieve to remove sand. Pieces of moss and twigs were picked out as much as possible, next the droppings were put in water to let small stone pebbles sink and then finally the droppings were dried again in an oven at 40^{o}C to enable prolonged storage without moulding. When the medium was

needed, droppings were ground in a blender with water in a mixture of 20g droppings and about 300 ml water. This was sometimes sterilised by cooking, and then 20 g/l dry yeast (*Saccharomyces cerevisiae siccum*) was added and the medium was stirred carefully with a spoon.

The "bad" medium consisted of water in which *Senecio* leaves and stems had been rotting for a few weeks. To this clear, yellow solution a few *Senecio* pieces were added both to prolong bacterial production, and to offer a surface for the larvae. This medium is "bad" for two reasons. The C/N atom ratio is quite high and hence limits growth of microorganisms on which the *Eristalis*-larvae rely for food. *Senecio* leaves contain different alkaloids, which are poisonous to a lot of animals and soluble in water. Alkaloids can have a direct negative impact on growth and development of *Eristalis* larvae, or indirectly via reduced microorganism numbers.

2.5 Rearing trays and containers

I planned to rear larvae individually, and others in groups. Groups were reared in the same white quadrangular trays as used by Ottenheim. These have two equal compartments of which one was filled with 100ml medium, and the other was supplied with a tissue and served as area for pupation. The transparent closing lid does not fit so tightly as to prevent airflow at any rate, but larvae are unable to crawl out.
To save room, the individual larvae were not reared in these large trays. We chose to use small round plastic containers (25 ml) with about 20 ml of medium, inside a larger (125ml) transparent container. The larvae crawl from the small tray in the bigger container and pupate there. The lids of these bigger round containers are secure, but were presumed not to be airtight. Unfortunately, they were, and all larvae reared in those trays died in the first *arbustorum* and *tenax* experiment. In the second *arbustorum* experiment, a number of small holes were made in the lids big enough to let in air but small enough to prevent larvae from escaping.

2.6 Experiments

The best way to present a study is often not in chronological order. However to understand the motivation behind decisions about details, the rearing bouts are presented here more or less chronologically.
To ensure that only one larva was transferred to an individual container, a larva was transferred to a petri dish with fresh clear water, where a check was made that it was really one. The dish stood over a black background to optimise visual clarity and contrast. This procedure was repeated with a second dish with fresh water, before the single larva was transferred to the individual container. Groups were transferred in the same way: the whole group was transferred three times and checked twice in the process.

2.6.1 Experiment 1: *Eristalis arbustorum*

This experiment was initiated in May with adults from Breda, Den Haag (Mariahoeve) and Leiden. For this rearing bout, the optimal medium was sterilized by cooking to prevent the possible criticism that any additional larvae could have come from eggs laid on the rabbit droppings in the dunes, however unlikely this may seem. A possible drawback might be that microbes are killed as well, but this was not expected to be problematic because I added 20g/l dry yeast after sterilising.

From the first few egg batches, 50% of the larvae were put in optimal rearing medium after hatching, and the other half in *Senecio* medium. About 50% of the larvae were assigned to a group of 10 and the remaining ones were put in individual containers. After the discovery of the mass mortality of the individuals in the small containers, some small experiments were done; individuals were put alone in the 100ml trays, and some small groups in small containers. The remaining egg batches were not

divided in four categories like the first ones, but assigned only to groups in trays with the optimal medium, in an attempt to rear as many larvae as possible.
Also, an experiment was performed where different media were tried in an investigation of the cause of the hundred percent mortality. Results will not be presented for the latter experiments concerning the media; paedogenesis never occurred. All in all, the mortality in the individual containers caused a lot of problems disturbing the neat design that was planned. Allocation to the different treatments is depicted in **table 2**. The trays were checked on pupae regularly the end of July, or until a change in the appearance of the medium indicated that no larvae were alive anymore.

2.6.2 Experiment 2: *Eristalis tenax*

This experiment was initiated in May with adults from Den Haag, Mariahoeve. All larvae were put in groups of 5 or 10 in 100ml medium, on June 11th. Twenty-four groups of 5 were initiated in rabbit dropping soup. The medium was not sterilized, but 9 containers with 100ml empty medium each served as controls. No larvae ever developed in these controls. A total of317 larvae were first reared in the *Senecio* soup and after 12 or 17 days the surviving larvae were translocated to the optimal medium. Of these, the remaining larvae of 12 groups of 5 were translocated on the 23rd of June. On the 28th, the larvae of 14 groups of 5, as well as those of 18 groups of 10 and 1 group of 7, were translocated. The treatments are depicted in **table 3**. The trays were checked regularly until they were discarded on the 21st of July.

2.6.3 Experiment 3: *Eristalis arbustorum*

This experiment was initiated in August with adult females from Leiden and Zwolle. Larvae were reared only individually. After hatching, the larvae were transferred in batches of about 50 larvae to trays with water and rotting plant cuttings of *S. jacobaea*. After a short period, usually of three days, larvae were transferred to individual containers with the optimal rearing medium of rabbit dropping soup with yeast, according to Ottenheim and Holloway (1995). The treatments are depicted in **table 4**. Initial numbers refer to the larvae allocated to the optimal medium. Mortality during the *Senecio* period was not routinely monitored, but was low in three days and variable but high in the batches that received the *Senecio* treatment for a longer period.
To check that no *Eristalis* larvae or eggs where already present in droppings from the dry coastal dunes, 10 control trays each with 500ml medium were set up. No larvae ever developed in these controls.
The containers were checked for pupae every two days from day sixteen to day twenty-four after hatching. The containers were thoroughly checked for any living or dead larvae before they were disposed of.

3 Results

During the study a total of 3300 small larvae were used, which resulted in 1148 successful pupations.

3.1 Experiments 1 and 2

In the first two experiments, no paedogenesis was found at all. In *Eristalis arbustorum*, (see **table 2**) survival in the optimal medium was moderately high and in the bad medium, or in the switch treatment, moderately low.
In *Eristalis tenax*, survival in the optimal medium was very high in comparison with *E. arbustorum*, but survival in the switch treatment was almost negligible. It appears that mortality was even higher in the individuals that remained in the bad medium longer. See **table 2** and **3**.
In the first experiment, with *E. arbustorum*, all individual larvae in the small containers died due to lack of oxygen. Corrected for this, the survival of *E. arbustorum* was slightly above 60 %.

group/indiv	tray	media	Senecio	initial no.	pupations	% survival	paedogenesis
group	large	D	0	512	398	78	
group	large	S	constantly	158	42	27	
group	large	SD	16	120	48	40	
individual	large	D	0	9	6	67	
group	small	D	0	213	0	0	
individual	small	S	constantly	257	0	0	
individual	small	D	0	328	0	0	
total:				1597	494	31	0

Table 2: Allocation of *E. arbustorum* larvae in the first experiment to the treatments with respect to group size, kind of tray and duration of *Senecio* treatment in days. S means only *Senecio* medium, D means dropping soup (optimal) and SD means a switch treatment with first *Senecio* medium and then dropping soup. The numbers refer to the number of larvae and not to the number of groups.

group/indiv	tray	media	Senecio	initial no.	pupations	% survival	paedogenesis
group5	large	D	0	120	103	86	
group5	large	SD	12	60	6	10	
group5	large	SD	17	70	1	1	
group10	large	SD	17	187	2	1	
total:				437	112	26	0

Table 3: Allocation of *E.tenax* larvae in the second experiment to the treatments with respect to group size, kind of tray and duration of *Senecio* treatment in days. S means only . D means dropping soup (optimal) and SD means a switch treatment with first *Senecio* medium and then dropping soup. The numbers refer to the number of larvae and not to the number of groups.

3.2 Experiment 3

1266 larvae of 16 families were used in the third experiment. Successful pupation occurred in 43% of the larvae, resulting in 545 pupae. Additionally, 110 larvae went into diapause and did not pupate before the end of the experiment. Including the diapauzing individuals, survival was slightly below 50 %, see **table 4**. Development time as such was not an object for this study, but it may be noted that development always took longer in animals that were subjected to a period of *Senecio* treatment. Five occurrences of paedogenesis were found, each time there were two individuals in the end, instead of one. The circumstances under which they occurred are summarized in **table 5**. No exact estimate of development time could be made. The development times of the paedogenetic larvae are shown in **table 5** and fall well within the normal range of larval development time, see **figure 4**. Here, the duration between the start of individual development in dropping soup and pupation are depicted, with arrows for the six known paedogenetic development times. In the last category, also the individuals still in diapause at the end of the experiment are counted.

batch	Senecio:	initial no.	pupations	diapauze	% survival	paedogenesis
La	3	89	36	0	40	
Lb	3	160	82	12	59	
Ma	3	180	79	18	54	
Mb	3	126	55	14	55	
Mc	3	70	21	7	40	2
Md	3	41	16	0	39	1
Me	3	96	40	5	47	
Mf	3	135	57	2	44	
Ra	3	42	10	10	48	
Rb	3	8	3	2	63	
Rc	3	15	4	3	47	
Lg	16	39	0	0	0	
Mj	18	24	1	5	25	
Rg	19	28	7	3	36	1
Mg	20	4	2	2	100	
Ld	21	23	18	2	87	
Me	22	57	30	15	79	1
Lb	6*	112	76	3	71	
Lb	6**	17	5	7	71	
total:		1266	542	110	52	5

Table 4: All larvae were treated individually in small containers, after a few days (indicated) of *Senecio* treatment in groups of about 50 individuals. Exceptions are: * after *Senecio*, five days in groups in optimal medium before individual initiation. ** after *Senecio*, ten days in a group in optimal, then thirteen days in tap water, and finally initiated individually. Numbers indicated are initial, pupated and diapauzing number of larvae. Diapauzing individuals are included in the survival, because they still have the opportunity to pupate. Five individuals engaged in paedogenesis, these individuals were from four egg batches.

Batch and individual code	*Senecio* treatment	Final stage	Development time (days)
Mc 50	3 days	Pupa	20
		Pupa	20
Mc 68	3 days	Pupa	24
		Large dead larva	-
Md 38	3 days	Small dead larva	-
		Large dead larva	-
Rg 8	19 days	Pupa	30
		Pupa	33
Me 112	22 days	Pupa	34
		Diapauzing larva	>48

Table 5: Summary of observed cases of paedogenesis. Each paedogenetic case resulted in two individuals and therefore are represented in two rows in this table. Development times are approximations of total development time, from hatching from the egg to pupation.

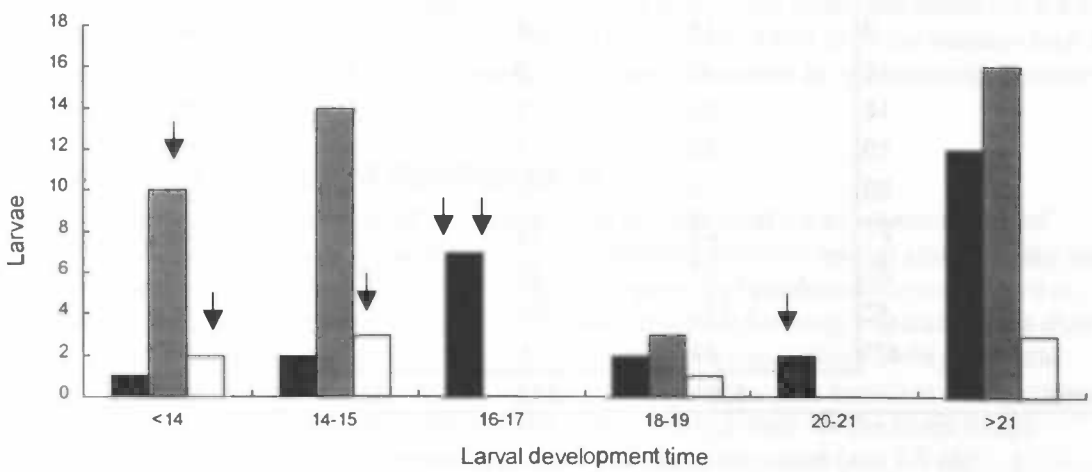
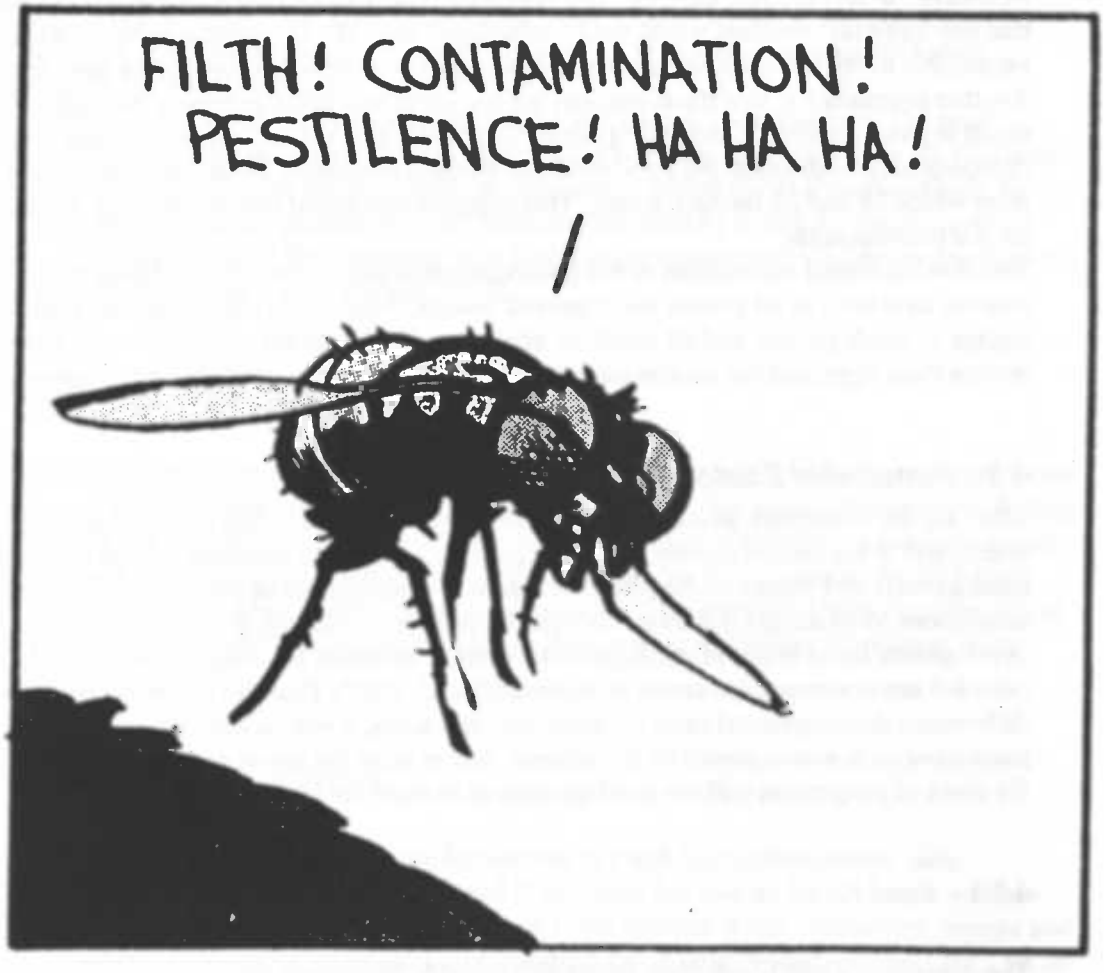


Figure 4: Histograms of the recorded larval development time in the good medium of the three relevant families, after transfer from *Senecio*. The arrows indicate the cases of paedogenesis in the different egg batches. Solid bars are Mc, grey bars are Me, and open bars are Rg.

3.3 Observations

The optimal medium is far from transparent and it is often difficult to find the larva in it at all. The larvae themselves are not transparent either due to their intestines. Because I wanted to rear as many larvae as possible to increase the number of paedogenetic cases, it was not feasible to carefully follow individual larvae throughout their development. Some observations however are of interest.
Two times I found an *arbustorum* larva that seemed dead, but "looked strange", and moved a very little bit upon touch with a needle. Both individuals, one in the first and one in the third experiment, developed large transparent cavities along their length axis immediately below the skin. They did not move, except when stung with a needle or when put in a hot light under the microscope. After a few days, they died. The question is whether those individuals were already dying when I discovered them, or maybe were on their way to paedogenesis.
Another important observation relates to the photograph of Ibrahim and Gad (1975). As a small experiment I put some larvae (10-13 mm) in clear tap water with a few dead prepupae. After twelve hours, some small larvae were inside the dead prepupae, eating their contents. Other prepupae had already been hollowed out. Finding small larvae inside a large one is therefore not sufficient proof of paedogenesis in *Eristalis*.



4 Discussion

4.1 Potential problems with the Eristalis results

This study shows that *E. arbustorum* larvae are able to reproduce and therefore document paedogenesis in *Eristalis*. Up to now we only found a doubling of the larvae but Ibrahim and Gad (1975) found two cases of three offspring from single *E. tenax* larvae.
The procedure of counting the larvae two times serves to eliminate the chance of putting in by mistake any additional larvae. An absolute guarantee that mistakes were never made cannot be given. All paedogenetic pupa and larvae, and all their siblings have been stored carefully to enable investigation of their genetic similarity with molecular techniques. Only this molecular work will provide strong proof for paedogenesis in *Eristalis*. If the reproduction is by apomixis, both individuals produced by paedogenesis will be genetically identical. If the reproduction is by automixis however, genetic similarity is lower, but is still expected to be higher than full sibs resulting from bisexual reproduction.
Larval development was not slowed down in the paedogenetic cases, which together with the finding of a small larva in container Md 38, could mean that the reproduction took place in an early stage. For example, paedogenesis might have happened immediately after the transfer from the *Senecio* to the optimal medium. The paedogenetic *E. tenax* larvae were however final instar (Ibrahim & Gad, 1975)
Under our study conditions, paedogenesis in *E. arbustorum* does not seem to occur often, as we found it only in 1 % of the larvae. We tried to induce paedogenesis by the bad/good medium switch treatment but this did not seem to work very well. The high mortality (see **table 2, 3** and **4**) suggests that the "optimal" medium might not be so optimal after all. The large number of trays made it impossible to stir the medium regularly, and fungus growth might sometimes have been a problem. Another possibility is that the containers are too small and paedogenesis would almost immediately result in overcrowding. Ottenheim (unpublished data) also observed paedogenesis in only rabbit dropping soup with yeast, the good medium. In these occasions 20 larvae were put in a tray of 200ml from which 28 and 23 larvae pupated. This suggests that larval interactions, e.g. via hormones, may induce paedogenesis.
The developmental mechanism of the paedogenesis is not known but it is likely to start from the ovaries, as it does in all known paedogenetic insects. This would also mean that males are not able to engage in paedogenesis and all resulting adults would be females. Adult *Eristalis* females take days to mature their eggs, and the acceleration in ovarian development needed in paedogenesis is remarkable.

4.2 Functional Ecology of Paedogenesis

What are the immediate advantages of paedogenesis to an individual (or to whatever unit of selection)? What factors govern its evolution? One hypothesis deserves special attention: the idea that paedogenesis and neoteny --despite their common consequence of paedomorphosis--evolve as adaptations to strikingly different ecological conditions: selection for early maturation and short development times result in paedogenesis, whereas selection for competitive ability in stable, crowded environments can result in neoteny (Gould, 1977). Because this hypothesis concerns differential developmental rates for germ-line and soma, it only counts for the cases where paedogenesis is accompanied by progenesis, as it is in all the insect cases (see 1.4 and 1.5), and also for cases of progenesis without paedogenesis as in most aphids.

4.2.1 r and K

The concept of r and K selection serves as a suitable framework for understanding the immediate

significance of progenesis and neoteny (Gould, 1977). The r denotes "the intrinsic rate of natural increase". Simply said, r selection predominates when the density-independent component of natural selection is in control--when populations can expand without negative feedback on growth rate by shortage of any resource. K selection will prevail when the density-dependent component dominates--when increase in one genotype must be at the expense of another (Gould, 1977). Attributes of r-selected organisms might include: high fecundity, greater proportion of available resources committed to reproduction, early maturation, short life span, rapid development and limited parental care. K strategists might employ low reproductive effort with late maturation, longer life, and a tendency to invest a great deal of parental care in small broods of late maturing offspring.

All attributes of gall midges and aphids, the best-known progenetic groups, point in the direction of r-strategies, except for their rather low fecundity. This is however exactly what is compromised by progenesis. Roff (1992) discusses a mathematical formulation of r determination. Depending on parameter values, accelerated maturation can be less, equally, or more effective than increased fecundity in raising r (Roff, 1992, p. 180-183).

4.2.2 Superabundant but ephemeral resources

Mushrooms and other fungi associated with specific stages of decay (for gall midges), the "red" stage in rotten wood (for *Micromalthus*) and young plant shoots (for aphids) are superabundant resources for the first individuals to colonize it. This superabundance dictates an r strategy, and progenesis provides it. The potential numbers of offspring of a single gall midge or aphid are impressive examples of animal reproduction. The paedogenetic phase can quickly build an enormous population in their ideal resource. The hypothesis seems justified: selection for early maturation and short generation times has led to paedogenesis.
However, the resources are intrinsically ephemeral in time and patchy in distribution. The rapid population increase must come to an end. Density dependence becomes important after all. It is here that a special attribute of progenetic insects comes in: they are so-called cyclical parthenogens. In almost all species, the progenetic, parthenogenetic phase is alternated by a mobile, non-progenetic bisexual phase. Upon crowding or food shortage, this bisexual generation provides an escape from the exhausted habitat and locates new ones (Gould, 1977).

4.2.3 Neoteny

On the other hand, neoteny is a possible result of K selection. Gould argues in relation to neoteny that " The selection is not, as in r situations, directly upon generation time, but upon a definite morphology required to maintain life in a stable environment." For the salamanders, a correlation has been found between the tendency to remain paedomorphic and both predictability of the aquatic, larval habitat and hostility of the terrestrial, adult habitat. Neoteny also seems to be correlated with complex social behaviour in K-selected mammals via prolonged neural development and longer learning periods (Gould, 1977).

4.2.4 Problems with r and K selection

These functional explanations, developed in the framework of r and K selection, sound quite reasonable. Unfortunately, the whole concept of r and K selection has lost its former credit and has been abandoned. It had great initial success in the 60's and 70's because it was convenient, simple and in accord with a popular explanation of population regulation, but it later proved incorrect for a lot of

reasons, listed by Stearns (1992). He advocates age-specific demographic models as a suitable framework to study the evolution of life history strategies.
As an example of this, Istock's treatise of the evolution of complex life cycles can be used (Istock, 1967). If larvae and adults receive a different proportion of total natural selection, the adult or the larval phase will be favored, leading to paedomorphism, or the elimination of the larval stage, or extinction (Istock, 1967).

4.2.5 Age or stage dependent selection

Paedogenesis without progenesis, as in sea stars, falls outside the realm of r-K strategies but could be seen as an extreme case of age dependent selection. The best guess is probably that paedogenesis has evolved as a strategy to do the best with available resources (Jaeckle, 1994). A larva unable to metamorphose successfully in its present environment must delay its transition. This situation occurs often in planktonic sea star larvae above the -unsuitable- ocean floor. The energy gathered during this delay cannot be invested in growth (above the optimal size), metamorphosis must be avoided, and here investment in reproduction seems a good alternative. This hypothesis is stated here in general terms because it might also count for other groups, e.g. insects during seasons unfavorable for adults but suitable enough for juveniles, or endoparasitic flatworms awaiting the predation of their intermediate host by their final host.

4.2.6 Sex

The advantage of paedogenesis is in the quantity of offspring. Because the process involves parthenogenesis in the insect cases, the genetic quality of the offspring could be at stake. Much has been said about the advantages and disadvantages of sex. A relatively new insight is that a little bit of sex might be an ideal compromise (Green and Noakes, 1995). Maybe, this compromise is reached in cyclical parthenogenesis, because a number of parthenogenetic generations are alternated by a bisexual one that has the hazardous task of locating new resources.

4.2.7 Evolutionary pathway towards paedogenesis in insects

Paedogenesis has evolved more than once in insects. Despite this, the cases show a remarkable similarity. The paedogenetic generations reproduce asexually. Paedogenesis is always a plastic character: under certain circumstances, a generation of normal sexual adults of both sexes will appear. This system is called cyclical parthenogenesis and occurs in aphids, the beetle *Micromalthus debilis* and in two subfamilies of gall midges. One hypothesis counting for all these cases is that first parthenogenesis evolved (Mamaev and Krivosheina, 1993). This seems to happen regularly and easily in many insect groups (Suomalainen *et al.*, 1987). Evolution of facultative parthenogenesis has to be accompanied by the evolution of a sex-determination system that allows production of both females and males through parthenogenesis. These two features give individual adult females the ability to lay eggs immediately upon hatching from the pupa, if the resource where she developed herself is still available. This immediate parthenogenetic reproduction can be advantageous if mating is time-consuming and bisexuality is more costly than parthenogenesis. However if the resource is exhausted, she has to disperse and locate a new one (and sex may now be advantageous because the environmental predictability is less).
The next steps in the evolution might have been progressively earlier onsets of oocyte and embryonic development in the part of the females predetermined -by the suitability of the environment- to stay

where they are and reproduce parthenogenetically. The case of *Paratanytarsus grimmii* (see §1.5.4) can be viewed as an example of an intermediate stage in this evolution. Metamorphosis will be selected against especially when it is time-consuming and the resource ephemeral. Progenesis can evolve gradually until larvae already develop their offspring inside, and oogenesis runs parallel with embryogenesis (Went, 1979, in Suomalainen *et al.*, 1987). During the evolution of earlier reproduction, specific adaptations are needed concerning the viviparity that accompanies paedogenesis.

This evolutionary picture is entirely hypothetical although it seems reasonable. A counter-case might be the absence of paedogenesis in Drosophilid flies. Many species use resources that are patchy and ephemeral, such as mushrooms and fruits. Parthenogenesis is well known in the group: one species, *Drosophila mangabeirai*, is entirely thelytokous and in several other species, unfertilized eggs can occasionally develop successfully and parthenogenetic lines have been established in the lab (Suomalainen *et al.*, 1987). Also, viviparity might not be particularly difficult to evolve: at least ovoviviparity exists, indicated by the observation that *Drosophila melanogaster* occasionally lays live larvae (Jan Sevenster, pers. comm.). The sex determination mechanism might be the most difficult part to evolve. With my level of knowledge it is also still possible that the resources are so short-lived that two generations simply take too much time.

Sometimes the evolution of paedogenesis might lead to total disappearance of the adult stage from the life cycle (Gould, 1977). Such species are not known in insects, which can be explained in part by the lack of knowledge of larvae in many insect groups. More important, such lineages have very low chances of escaping from extinction when the resources are still ephemeral and larvae are unable to disperse to new resources. *Micromalthus debilis*, especially the South African populations, could be seen as an example of an intermediate step on this evolutionary pathway. The active first stage larvae can serve as the major dispersal stage. When the adult stage is abandoned, mutations can accumulate in the part of the genome used only in the adult (Martin and Gordon, 1995). In this respect it is of interest to note the aberrations that have been found in South African females of *Micromalthus*. Most individuals have seven free abdominal segments instead of six as in the American specimens. But in some African individuals, although seven tergites are observed, only six sternites are visible, the sixth being the largest and obscuring the reduced seventh (Paterson, 1938).

4.2.8 Implications for *Eristalis*

The importance of paedogenesis in the life history of *E. arbustorum* is not clear. The most promising hypothesis seems to be related to situations where r selection would prevail: when very suitable but ephemeral habitats are encountered, e.g. cattle dung, large amounts of grass cutting or dead animals rotting in water. These environments might enable several larval generations before microorganism densities become too low for rapid development. Paedogenesis could also be advantageous under conditions where adults cannot function but larvae can; for example outside the flight period, from September to March. The advantage critically depends on mortality, fecundity and energy balance in the different stages in the field, but these data are lacking.
Paedogenesis might be advantageous under some of the above-mentioned conditions, but this alone is not enough for it to function. Larvae must also be able to make predictions about the future. This would for example mean that they should be able to monitor microorganism densities (or correlates of this quantity) in the present and estimate those in the near future. This would enable a "rule of thumb" for engaging in paedogenesis. Alternatively, it can also be argued that paedogenesis in *Eristalis arbustorum* is not adaptive but a rare accident, perhaps comparable to identical twins in humans.

5 Acknowledgements

Many helpful persons were important during this fascinating project. First I want to warmly thank my supervisors Mart Ottenheim, Leo Beukeboom and Paul Brakefield for their continued enthusiasm for the project and for sharing their knowledge and insights. Mart's renewed occupation at the institute in June was a big share of luck, for he was to be my closest supervisor. I especially thank Mart for always having time and good criticism even when I didn't ask for it. V. S. van der Goot has been essential in this project: he provided Mart with the reference to the work of Ibrahim and Gad, he wrote the short note in "De Vliegenmepper" that first attracted my attention to the subject and he helped with general entomological literature. I am very grateful to Wijnand Heitmans, Antonia Monteiro, Bas Zwaan, Freerk Molleman, Hans Roskam, Roy van Grunsven, Jan Sevenster, and Vernon French for helpful comments. Peter Korsten, Gé Boskamp, Coenraad Krijger, Herman Berkhoudt and all the people of Evolutionary Biology provided company and all kinds of help. Rohan de Groot, Eric Thomassen, Railys Jamanika, and Gerben Achterkamp assisted in dirty practical work. Thanks!

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7 Suggested literature

More literature about paedogenesis and related subjects is listed here. A lot of the references are redundant with the information brought together in my report, but some references promise very interesting additional information. I just had too little time to lay my hands on the articles, or to read them. Some journals in this list are abbreviations of journals not known to me. Also some abbreviations could be false, which could explain why I was unable to find them. (The most extreme case of this was "Z. n. Z." for "Zeitschrift fuer wissenschaftliche Zoologie" in Carpenter, 1928).

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Appendix: the letter to Ibrahim and Gad.

Leiden, 9 November 1999

Dear Dr. Gad, dear Dr. Ibrahim,

At the moment I am involved in a project that is more or less successive of your work on *Eristalis*. During a study on plasticity of colour in *Eristalis arbustorum*, by my supervisor Dr. M.M. Ottenheim, there was found on two occasions that more prepupae were crawling from the medium than the number of larvae he had put in. Your article (1975: J.Med.Ent. 12, 2: 268) gave the explanation: paedogenesis. I am studying under what conditions this extremely interesting phenomenon does occur in *Eristalis arbustorum*.
In relation to this I would like to ask you a few questions.

1) What rearing medium did you use? Was it just water, or did the water contain something else?
2) I was able to induce situations like the one you photographed, by placing young larvae in ordinary tap water with a few full-grown, dead larvae. The small ones simply crawl into the rotting body via mouth or anus. This means that your photograph alone does not convince critics, of which there are many! Then your eight series of five larvae become very important. The J.Med.Ent. article does not give details about the rearing pans. How were the pans closed? Can you exclude the possibility that the three small larvae could have come in by accident, or form another pan, as critics might remark?
3) What was the source of the larvae? Did you catch larvae outdoors, or did you obtain larvae from eggs laid by captive females? There is a critic who doubts that the species was *Eristalis tenax*. Are you sure about the species determination?
4) Did you publish other articles about *Eristalis*, or is it published in a separate Ph.D. thesis? If so, Dr. Ottenheim and I would like to have a copy. Is it possible for you to send a copy? Of course we are willing to pay for your expenses.

In December we will present our results in a talk on the yearly symposium of the Dutch Entomological Society. This will also be published in the Proceedings of this symposium. If you would like to have a copy, I would be happy to send you a pre-publication in December.

I would be extremely grateful for your reaction. As I wasn't sure about your present address, I have asked a few of your recent co-authors to forward this letter to you. I just hope I didn't bother you too many times.

Sincerely Yours,

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