

TRIPLOIDY IN THE NATURAL POPULATION OF THE
PSYCHODINE MOTH FLY *PSYCHODA PARTHENOGENETICA*
TONNOIR (DIPTERA: PSYCHODIDAE)

G. TROIANO

Istituto di Zoologia, Università di Genova, Genova, Italy

Received: 19th July 1977

INTRODUCTION

Thelytoky is a fairly well-known phenomenon within the Diptera. It was found in a number of species belonging to different families, although more frequently among the Chironomidae (SCHOLL 1956, 1960; LINDBERG 1958; PORTER 1971) and Simuliidae (BASRUR and ROTHFELS 1959). Within the Psychodinae, besides *Psychoda parthenogenetica*, VAILLANT (1971) reports three other species, *Psychoda phalenoides*, *P. lativentris* and *P. moravica*, in which this mode of reproduction seems to be present. Therefore on the whole the tendency to thelytoky seems to be a characteristic typical of the genus *Psychoda*. In fact no case of parthenogenesis was found in other genera of the subfamily Psychodinae. Unfortunately none of the parthenogenetic forms have been studied till now from a cytological standpoint, in spite of the interest in such an analysis.

Different conditions of thelytoky occur among the Diptera. Nevertheless several species show a quite similar condition, having a triploid complement and a type of apomictic thelytoky. This same condition has been found in *P. parthenogenetica*.

A cytological analysis of *P. parthenogenetica* appeared interesting, apart from reasons of a general character, because it could advance a substantial contribution to the solution of an old taxonomic problem relative to this species. Indeed, a bisexual species exists, *Psychoda severini*, the females of which differ little or not at all from *P. parthenogenetica*. Moreover the two species have ranges which overlap widely, at least in Europe, and often coexist in the same biotope. For this reason authors (SARÀ 1950; SALAMANNA 1975) believed they were the same species and supposed that the males, which besides were not found frequently and always in small numbers,

could arise parthenogenetically. Other authors (TONNOIR 1940; VAILLANT 1961; DUCKHOUSE 1962), on the contrary, believed that sufficient morphological differences exist between the two forms to regard them as separate species. Such an opinion would furthermore be supported by the results of the breeding of *P. parthenogenetica* which, although prolonged only for few generations, have only produced females. These results nevertheless have been considered not quite decisive owing to the artificial conditions of the laboratory. The whole history of this debated question is reported in a recent paper of SALAMANNA (1975). Although a parthenogenetical generation of males is an unlikely event, which has been reported only once within the Diptera (BASRUR and ROTHFELS 1959), it is plain that the taxonomic problem exists. Only it has been stated perhaps wrongly. The present study, I think, puts the problem back in the right terms and may constitute a basis for its definitive solution.

MATERIAL AND METHODS

Psychoda parthenogenetica is a widely diffused species (Europe, Algeria, Japan, Korea, New Zealand, Australia and many little oceanic islands). It may be rather easily found in all those environments where there is stagnant water or dripping from rocky walls which are the habitat common to most of Psychodinae. The larvae feed on rotting vegetable matter, but very likely on other organic materials also, even artificial ones such as paper, where I have actually found it. These alimentary practices have brought the species into domestic environments which may have facilitated somehow its diffusion. The species has been bred in the laboratory beginning from a few larvae collected at Prato, near Genoa. The culture medium was boiled minced lettuce. The species breeds easily and does not require any particular treatment. In laboratory conditions the biological cycle lasts on the average 20 days, 15 of which are spent in the larval state and 5 in the pupa. Till now we have obtained five generations and no males have been found.

The brain and ovaries were the materials used for the cytological analysis. These last were drawn from larvae at IV stage or prepupae to observe the meiosis, if present. From these same larvae the salivary glands were drawn for the polytene chromosome study. The ovaries are subspherical and very small. Their size is inferior to those of other psychodid species observed, both in the relative and the absolute sense. The same is true of the salivary glands. The larvae were dissected in a 9:5:6 mixture of glacial acetic acid, 85% lactic acid and distilled water. The brain or ovaries were then placed in a drop of 1% acetic carmine and squashed between a slide a coverslip after about one minute. The salivary glands were stained by 1% acetic orcein. Analysis and photography of the preparations were made with a Leitz Hortoplan Photomicroscope in phase contrast and with a green filter.

RESULTS

In all the metaphase plates both of brain and ovaries one may observe fifteen chromosomes often arranged in groups of three (Figs. 1, 2). This arrangement is very evident in the oogonial metaphases and especially in the maturative ones in which the chromosomes are on the whole less thickened and the different groups may be more easily distinguished (Figs. 3, 4, 5). Therefore, in this case also the somatic pairing phenomenon, typical of the Diptera, is evident. Nevertheless we could seldom observe a close chromosome synapsis (Fig. 6), such as the one found in *Pericoma modesta* (TROIANO 1977); whereas in some cases a more or less incomplete synapsis has been found (Fig. 7). The reconstruction of the different triads is possible even if the chromosomes are more or less scattered since the elements of each group differ in shape and size from these of the other groups. In fact, on the grounds of the length, one may distinguish, at first sight, two largest but non equal triads, two intermediate, these also unequal, and one smallest. The chromosomes are all metacentric or submetacentric. Heterochromosomes have not been found.

A karyometric analysis of the species has been made upon the system proposed by LEVAN *et al.* (1964), arbitrarily numbering the chromosomes from I to V, beginning from the largest. The relative measurements listed in Table I refer to Fig. 5. Unfortunately this type of analysis has not been made also in the species of *Psychoda* observed by SARÀ (1949), i.e. *P. alternata* and *P. cinerea*, so that a precise comparison of the three complements is not possible. Nevertheless, on the basis of the observations of this author, the karyotypes of the three species seem to be quite similar. Indeed SARÀ (1949) observed in the spermatogonial metaphases both of *P. alternata* and *P. cinerea* five pairs of chromosomes having median or submedian centromere. He distinguished moreover a largest pair, two intermediate but not equal and two smallest pairs. The chromosomes are all very small (2-4 micron); heterochromosomes are not present. Therefore the genus *Psychoda* seems to have a considerable uniformity of the karyotype, which besides accords with the morphological evidence.

The thelytoky of *P. parthenogenetica* is apomictic in type as we may expect in a triploid species. No trace of meiosis has been observed even abortive in type, such as the one found in some thelytokous species of Chironomid midges (SCHOLL 1956, 1960; PORTER 1971). The maturation of the oocytes is achieved by simple mitotic divisions. The oogonial and maturative metaphases appear therefore fairly similar. Nevertheless they may be rather easily distinguished by the relative size of the cells and

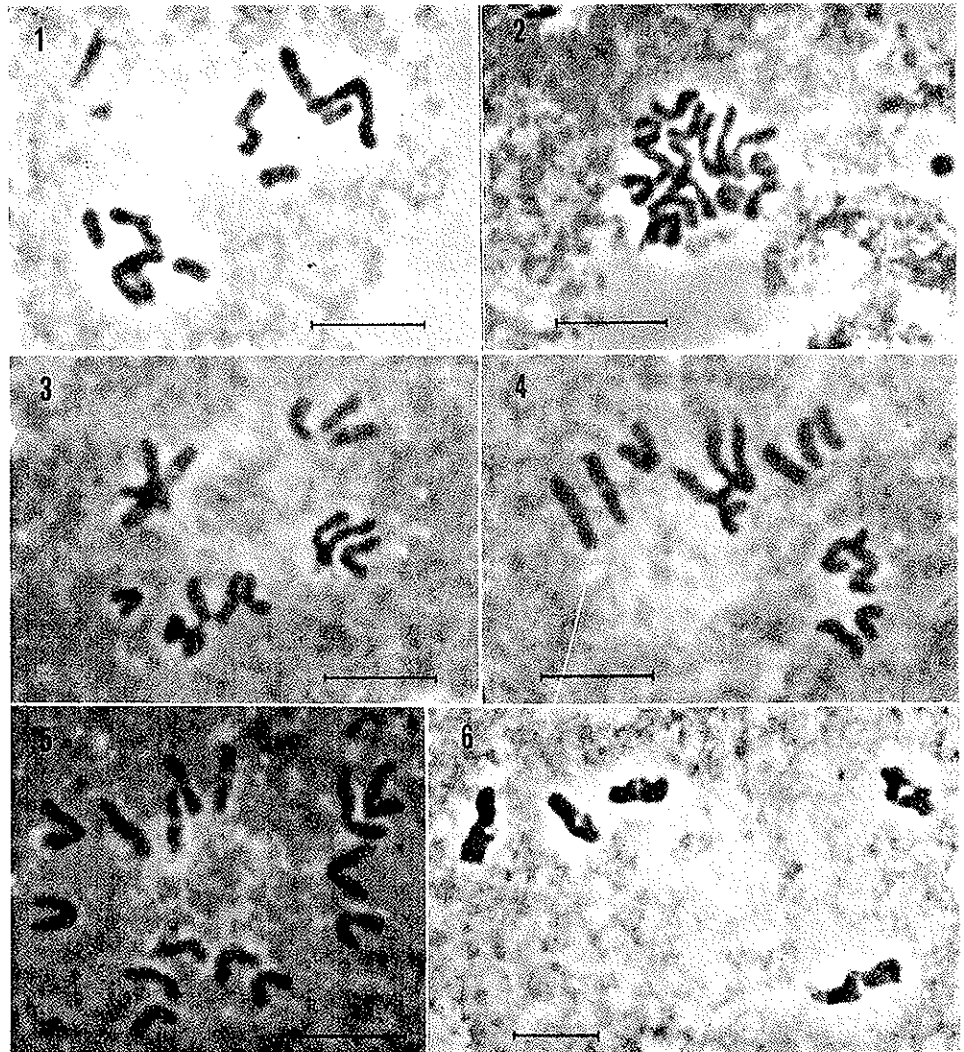


Fig. 1. — Mitotic metaphase from brain cells.

Fig. 2. — Oogonal prematurative metaphase. Note the typical arrangement in triads of the chromosomes.

Figs. 3, 4, 5. — Maturative metaphases showing the different chromosome triads.

Fig. 6. — Maturative metaphase showing a close chromosome synapsis. Scales represent 5 micron.

chromosomes and by the position of the same cells in the ovary, whenever it is possible.

As regard the polytene chromosomes, unfortunately in the present species they are not suitable for a detailed study such as the ones of other Psychodid species (AMABIS and SIMOES 1972; TROIANO 1975, 1977). Both

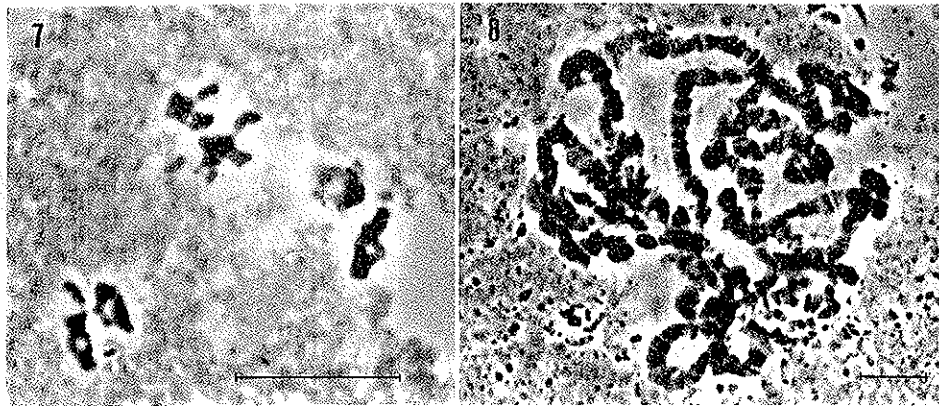


Fig. 7. — Maturated metaphase showing an incomplete chromosome synapsis.

Fig. 8. — Polytene chromosomes from salivary gland cells. Scales represent 10 micron.

the salivary glands and Malpighian tubules have been employed to this end but always bad results have been obtained. The cause consists principally in the difficulty of the chromosome spreading, which probably is due partly to the relative smallness of the cells and partly to the triploidy which produces a more close 'packing' of the chromosomes in the nucleus. This is a great handicap which does not let us observe the presence of an eventual chromosome polymorphism, as the one found in other thelytokous Diptera, nor to make detailed comparisons both between different populations of the thelytokous form and between this and the related bisexual form.

TABLE 1
Karyometric analysis of P. parthenogenetica.

Chromosome no.	c	s	l	i	d	r
I	16,8	7,3	9,5	43,45	2,2	1,30
II	14,4	6,3	8,1	43,75	1,8	1,29
III	12,6	6,2	6,4	49,21	0,2	1,03
IV	12,0	5,1	6,9	42,50	1,8	1,35
V	11,3	5,3	6,0	46,90	0,7	1,13

c = total length of chromosome; s = length of short arm; l = length of long arm; i = centromeric index (ratio between arms) ($i = 100 s/c$); d = location of centromere; ($d = l-s$); r = ratio of arms ($r = l/s$). (after LEVAN *et al.* 1964).

DISCUSSION

The definition of the karyotype and the type of thelytoky present in *Psychoda parthenogenetica* while it clarifies some problems relative to this species, leaves many others remaining to which we cannot give an exhaustive

answer for the moment. A first series of questions concerns the true relationships existing between *P. parthenogenetica* and *P. severini*. The triploidy found in *P. parthenogenetica* seems to confirm a complete thelytoky and hence the existence of two distinct forms, one thelytokous and the other bisexual. In fact only one possible alternative exists which could deny this affirmation, that the males be occasional triploid individuals generated by parthenogenesis, as it seems to occur in a species of *Prosimulium* (BASRUR and ROTHFELS 1959). This eventuality, however unlikely, cannot be excluded for the moment. Nevertheless, granting the existence of two distinct forms, their phylogenetic interrelationships remain to be ascertained. At the moment it seems rather evident that the two forms or supposed species are closely related in the sense that the thelytokous form directly arose from the bisexual one. The geographical distribution seems to confirm, rather than refute, this hypothesis. In fact the thelytokous form is widely diffused, whereas the bisexual is present only in Western Europe where evidently the two forms coexist, often dividing the same biotope. The wide geographical distribution of the thelytokous form is very likely due to its greater capacity for adaptation and mostly for dispersion in comparison with the bisexual one. This latter moreover shows a discontinuous and apparently residual distribution. This may be attributed to the same genetic and biological causes which have produced the origin and successful development of the thelytoky, and perhaps to the competition of the same thelytokous form, at least at beginning. Anyhow the coexistence of the two forms on a wide range makes one think of a case very similar to the one of the simuliid *Cnephia mutata* (BASRUR and ROTHFELS 1959), a 'super-species' which includes two forms, a diploid bisexual one and a triploid thelytokous one. In this case also the two forms frequently coexist in the same biotope, but there is good evidence that they do not interbreed. In conclusion *P. parthenogenetica* and *P. severini* could be regarded as separate species, in the sense of morphospecies, if only evident morphological differences existed between the two forms. On this point the matter is in the hands of the students specialised in the taxonomy of this family. Nevertheless I would add that whenever thelytokous forms are proper species, in the aforesaid sense, such as the mantid *Brunneria borealis*, the tettigoniid *Saga pedo*, the grasshopper *Warramaba virgo* and *Drosophila mangabeirai*, they are geographically isolated from the related bisexual or these are extinct.

Another series of questions concerns the origin both of the thelytoky and triploidy. Personally, I believe *P. parthenogenetica* is of allopolyploid and polyphyletic origin. Concerning the thelytoky, considering that the genus *Psychoda* shows a strong tendency to this type of reproduction, it is easy to think that the thelytoky of *P. parthenogenetica* is not an occasional event

but it must have occurred in several occasions and in different populations. The triploidy may have arisen afterwards in the same way, by occasional meetings between females of the thelytokous form and males of the related bisexual one. The primary diploid thelytokous form should have been then replaced by the genetically more versatile triploid. Nevertheless this does not exclude the possibility that diploid thelytokous populations may still survive somewhere. A polyphyletic origin could fully explain the wide diffusion of *P. parthenogenetica*. In fact an eventual heterozygosity present in the primary bisexual form could have given origin in this way to a more or less great number of genetically different biotypes or cytotypes capable of adapting themselves to different ecological niches. The heterosis could have been increased by the following triploidy. In that case the supposed ecological versatility of *P. parthenogenetica* should truly be attributed to the existence of a genetically polymorphic complex. On the other hand the presence of a chromosomal polymorphism has been found in many thelytokous 'species' of Diptera (BASRUR and ROTHFELS 1959; SCHOLL 1956, 1960; PORTER 1971; CARSON *et al.* 1957; CARSON 1962; MURDY and CARSON 1959; BLOCK 1969; STALKER 1956*a*, 1956*b*), even if in some cases it seems it may have arisen by following divergent evolution.

It is plain that a lot of the hypotheses so far expounded are founded rather on considerations of general character than on real cytological and morphological evidence. A definitive answer will be given only by further biological, morphological, especially biometric, and cytological investigations into the two 'species' and in different populations. Unfortunately, the unavailability of the polytene chromosomes sets limits to our possibilities of analysis and hence of the solution the problems relative to this species.

REFERENCES

- AMABIS J. M. and SIMOES L. C. G., 1972. — *Chromosome studies in a species of Telmatoscopus sp.* Caryologia, 25: 199-210.
- BASRUR V. R. and ROTHFELS K. H., 1959. — *Triploidy in natural populations of the black fly Cnephia mutata (Malloch).* Can. J. Zool., 37: 571-580.
- BLOCK K., 1969. — *Chromosome variation in the Agromyzidae. II. Phytomyza crassiseta Zetterstedt—a parthenogenetic species.* Hereditas, 62: 357-381.
- CARSON H. L., 1962a. — *Fixed heterozygosity in a parthenogenetic species of Drosophila.* Studies in Genet. II, Univ. Texas Publ., 6205: 55-62.
- CARSON H. L., WHEELER M. R. and HEED W. B., 1957. — *A parthenogenetic strain of Drosophila mangabeirai Malogolowkin.* Univ. Texas Publ., 5721: 115-122.
- DUCKHOUSE D. A., 1962. — *Some British Psychodidae (Diptera, Nematocera): description of species and a discussion on the problem of species pairs.* Trans. R. Ent. Soc. London, 114: 403-436.
- LEVAN A., FREDGA K. and SANDBERG A. V., 1964. — *Nomenclature for centromeric position on chromosomes.* Hereditas, 52: 201-220.

- LINDBERG B., 1958. — *A parthenogenetic race of Monotanytarsus boreoalpinus* Th. (Dipt. Chironomidae) from Finland. Ann. Ent. Fenn., 24: 35-38.
- MIROUSE R., 1942. — Sur l'existence d'une parthénogénèse constant thélytoque chez *Psychoda severini* Tonnoir (Diptères). C.R. Acad. Sc., 215: 226-227.
- MONTALENTI G., 1946. — Sui cromosomi e il numero dei chiasmi nella spermatogenesi di *Psychoda* sp. (Dipt. Psychodidae). Rend. Accad. Naz. Lincei, Serie VIII, I: 120-122.
- MURDY W. H. and CARSON H. L., 1959. — Parthenogenesis in *Drosophila mangabeirai* Malog. Amer. Nat., 93: 355-363.
- NARBEL-HOFSTETTER M., 1964. — Les Altérations de la Méiose chez les Animaux Parthénogénétiques. Protoplasmatologia VI/F/2, 190 pp. Vienna: Springer-Verlag.
- PORTER D. L., 1971. — Oogenesis and chromosomal heterozygosity in the thelytokous midge, *Lundstroemia parthenogenetica* (Diptera, Chironomidae). Chromosoma, 32: 333-342.
- SALAMANNA G., 1967. — Biologia riproduttiva di *Telmatoscopus albipunctatus* Will. in condizioni di laboratorio (Dipt. Psychodidae). Boll. Zool., XXXIV: 170. Atti del XXXIV convegno dell'U.Z.I.
- , 1975. — Psychodidae Psychodinae della Puglia e della Basilicata con descrizione di due nuove specie (Diptera Nematocera). Entomologica, Bari, XI: 193-214.
- SARÀ M., 1949. — Sulla spermatogenesi di *Psychoda alternata* Say e di *Psychoda cinerea* Banks. (Dipt. Psychodidae). Sci. Genet., III: 1-11.
- , 1950. — Sui cromosomi di *Telmatoscopus albipunctatus* con alcuni dati su quelli di *Telmatoscopus ustulatus* (Diptera: Psychodidae). Caryologia, 3: 204-210.
- , 1950. — Psicodidi dell'Italia centro-meridionale e descrizione di una nuova specie di *Telmatoscopus* (Dipt. Nematocera). Ann. Ist. Mus. Zool. Univ. Napoli, 2: 1-8.
- SCHOLL H., 1956. — Die Chromosomen Parthenogenetischer Mücken. Naturwiss., 53: 91-92.
- , 1960. — Die Oogenese einiger parthenogenetischer Orthocladiinen (Diptera). Chromosoma, 11: 380-401.
- STALKER H. D., 1956a. — A case of polyploidy in Diptera. Proc. Nat. Acad. Sci. U.S.A., 42: 194-198.
- , 1956b. — On the evolution of parthenogenesis in Lonchoptera (Diptera). Evolution, 10: 345-359.
- TONNOIR A. L., 1940. — A synopsis of the British Psychodidae (Dipt.) with description of new species. Trans. Soc. Brit. Ent., 7: 21-64.
- TROIANO G., 1975. — The salivary gland chromosomes of *Telmatoscopus albipunctatus* (Williston) (Diptera: Psychodidae). Caryologia, 28: 321-333.
- , 1977. — Chromosome studies in *Pericoma modesta* Tonnoir (Diptera: Psychodidae). Caryologia, 30: 27-39.
- VAILLANT F., 1961. — Révision des Psychodidae Psychodinae de France (Diptera). Ann. Soc. Ent. France, 130: 131-157.
- , 1971/75. — Psychodidae Psychodinae, In: E. Lindner. Die Fliegen der palaearktischen Region, 9d, pp. 1-182.
- WHITE G. B. and KILLICK-KENDRICK R., 1975. — Polytene chromosomes of the sandfly *Lutzomyia longipalpis* and the cytogenetics of Psychodidae in relation to other Diptera. J. Ent., 50: 187-196.
- WHITE M. J. D., 1973. — *Animal cytology and evolution*, Third Edition, 961 pp. Cambridge University Press.

SUMMARY

A cytological analysis of the thelytokous species *Psychoda parthenogenetica* has been made. A $3n=15$ complement and a type of apomictic thelytoky have been found. Apart from the triploidy, the complement of *P. parthenogenetica* is very similar to the one of *P. alternata* and *P. cinerea*. The phylogenetic interrelationships with the related bisexual species *P. severini* and the origins both of the thelytoky and triploidy have been discussed.