

Esser, K. An introduction to

Podospoma anserina.

In order to introduce *Podospora* genetics to those scientists who are not able to read the original papers, mostly written in French or German, we would like to describe briefly the culture methods and the problems studied with this fungus. A stock list containing the seven linkage groups is included for further information.

The ascomycete *Podospoma anserina* belongs to the family Sordariaceae and is therefore very closely related to *Neurospora* and *Sordaria*, which belong to the same taxon. Formerly, many synonymous names have been used for *Podospora*: *Sphaeria*, *Plaurage*, *Bombordio*, *Sordaria*, *Schizothecium*, *Schizotheca*, *Philocopra*. However, in recent years the name *Podospora* has succeeded there. Whereas the older papers, published around 1930 by Dowding, Ames, and Dodge, dealt mainly with ontogenesis and morphology, the work of Rizet created the formal genetics of *Podospora* (Rizet and Engelmann 1949 Rev. Cytol. Biol. Vegetales 11: 201).

1. Life cycle and genetic peculiarities. The natural habitat of *P. anserina* is the dung of herbivores. Most strains have been isolated from horse dung in France and Germany. Its life cycle follows essentially that of *Neurospora* with a few exceptions: the mycelium grows somewhat like *Neurospora* colonial types, with a growth rate of 7 mm/d at 27°C. There are no macroconidia formed but only microconidia which, however, generally do not germinate. They act as male gametes and are able to fertilize the ascogonia via a trichogyne and therefore are called spermatia. The asci develop only four spores, which contain in the beginning of spore development two non-sister nuclei of the post-meiotic mitosis; therefore, each spore contains the genetic information of half a tetrad. Due to this mechanism, spores are heterocaryotic for all factors which exhibit second division segregation and, for tetrad analysis, it is not necessary to isolate the spores in order.

The size of these spores is 37 x 19 µm; they are considerably larger than the ascospores of *N. crassa* (28 x 14 µm). The mating behavior of *P. anserina* is controlled (like in *N. crassa*) by the bipolar mechanism of homogenic incompatibility leading to two different hermaphroditic mating types called + and -. Since the +/- alleles have a post-reduction frequency of about 98%, the normal binucleated spores are heterocaryotic for the mating type alleles. The mycelia originating from normal spores are mostly self-fertile, due to the fact that the + ascogonia may be fertilized by the - spermatia (and vice versa), both being present in the same mycelium. This phenomenon, also known in *N. tetrasperma* and *Gelasinospora tetrasperma*, has been called pseudocompatibility (secondary homothallism). However, in about 1-2% of asci of most strains (especially in young perithecia), instead of one binucleated spore a pair of spores is formed, each originating from a single nucleus. The mycelia growing from these spores are self-sterile, having either mating type + or -, and act, concerning their mating behavior, like *N. crassa* mating types. In analysing these (at least) 5-spored asci, tetrad analysis can easily be performed. For details of the life cycle and formal genetics of *Podospora* see the following papers: Beisson-Schecroun, J. 1962 Ann. Genet. 4:4; Bernet, J. 1965 Ann. Sci. Not. Botan. Biol. Vegetale 6:611; Esser, K. 1956 Z. Vererbungslehre 87:595 and 1959 Z. Vererbungslehre 90:29 and 445; Esser, K. and R. Kuenen 1967 Genetics of Fungi. Springer, New York. pp. 500; Kuenen, R. 1962 Z. Vererbungslehre 93:35 and 66; Marcou, D. 1961 Ann. Sci. Not. Botan. Biol. Vegetale 2:653; Rizet, G. and C. Engelmann 1949 Rev. Cytol. Biol. Vegetales 11: 201.

2. Culture methods and technical advice.

Minimal medium #1: (modified Westergaard medium) contains in 1000 ml solution: 20 ml mineral concentrate, 20 g fructose, 200 µg thiamin. The solution is brought up to pH 6.4-6.6 with 10% KOH solution (about 2 ml) and 20 g Bacto-Agar is added if solid medium is desired.

Mineral concentrate contains in 1000 ml: 25 g MgSO₄ x 7H₂O, 5 g NaCl, 5 g CaCl₂ x 6H₂O, 50 g KH₂PO₄, 50 g KNO₃, and 5 ml of trace element concentrate.

Trace element concentrate contains in 100 ml solution: 5 g Ascorbic acid x 1H₂O, 5 g ZnSO₄ x 7H₂O, 1 g Fe(NH₄)₂-(SO₄)₂ x 6H₂O, 0.25 g CuSO₄ x 5H₂O, 0.05 g MnSO₄ x 1H₂O, 0.05 g H₃BO₃, and 0.05 g Na₂MoO₄ x 2H₂O.

The mineral and the trace element concentrates may be stored at room temperature; in order to avoid contamination, a few ml of chloroform may be added.

Minimal medium #2: contains in 1000 ml: 0.25 g KH₂PO₄, 0.3 g K₂HPO₄, 0.25 g MgSO₄·7H₂O, 0.84 g NaNO₃, 2.5 µg biotin, 50 µg thiamin, 6 g fructose (purified on activated charcoal) and 0.1 ml trace element concentrate.

Complete medium: (modified Rizet's medium): To 1 liter of corn meal extract is added 1.5 g malt extract and 20 g agar. To obtain corn meal extract, 250 g corn meal is extracted in 10 liters of water and kept at 60°C over night. Thereafter, the solution is cautiously poured off and the remaining corn meal is discarded. The commercially available Difco corn meal agar may be used or well.

Defined complete medium: has the same composition as minimal medium 2 except that NaNO₃ is replaced by urea (0.5 g/liter) and fructose is replaced by yellow dextrin (10 g/liter). The life cycle is better achieved on this medium when the humidity is high (70%).

Moist horse dung: is used for crossing some very weak mutant strains

Spore germination medium: consists of 0.44% Ammonium acetate in complete medium, or a 1 % solution of Bacto-peptone, or a 1 % fructose solution (for detecting nutritional mutants).

Mass production of microconidia medium: contains in 1000 ml; 2 g sorbose, 2 g yeast extract, and 1 g glucose.

For biochemical work the fungus can be cultivated in aerated liquid medium. In a 10 liter carboy we obtain, after 4-5 days of incubation in complete medium, about 50 g of mycelium wet weight (mycelium pressed dry between filter paper).

Since *Podospore* spores do not have a dormancy phase, spore germination can be observed a few hours after inoculation. Spore germination occurs also on the other media mentioned above and even in water droplets. However, a spore germination rate of about 95%. at least, can only be obtained on the spore germination medium. The fructification of *Podospore* can be markedly enhanced by light. Open shelves in a culture room or incubators with glass windows which allow entrance of the rays of a simple fluorescent light tube are sufficient. The optimal culture temperature is 27°C. Under optimal conditions, the life cycle of *Podospore* is completed in 8-15 days, depending on the strain and the medium.

Crosses are performed either by confrontation of the monocaryotic mycelia or by spermatization (pouring a suspension of microconidia (filtered on fritted glass, 10-20 μ pore size) over a monocaryotic mycelium used as the female parent). This latter technique allows genetic analysis of dicaryotic self-fertile strains and comparison of the reciprocal crosses.

Spores are isolated under a dissecting microscope. To start this procedure, ripe perithecia are cracked with a watchmaker's forceps and the contents, with asci in all ranges of maturity, are transferred to a 5 % water-agar. With some practice the asci may be dissected with small steel pins or steel needles and isolated.

Moss spore isolations also may be performed in the following way: from cultures with ripe perithecia growing in Petri dishes, the lid is removed and replaced by a Petri dish filled with 2-5 % water-agar. Keeping them below the light source will provoke the perithecia to shoot their asci against the cover plate. In most cases the four spores of a tetrad stick together. The shooting procedure may be enhanced by tapping the plates gently on the table after illumination for one hour.

Protoplasts can be easily obtained by Bachmann and Bonner's method and conveniently freed from all living mycelium fragments by filtration through fritted glass filters (20-40 μ).

Stock cultures may be kept on all kinds of solid media (excepting the germination media) in the refrigerator at 4°C.

5. Strains and linkage-groups.

All the mutant strains were obtained from (or isogenized with) the same geographic race, first isolated and described by Rizet (1952 Rev. Cytol. Biol. Vegetales 13:51), bearing either the gene *S* or its allele *s*. Corresponding to the 7 chromosomes of *Podospore*, there are seven linkage groups. After the description of the located mutants we will briefly mention the genes not yet mapped. With the exception of some spore (size, color, shape) mutants, which are named with numbers, the morphological mutants have been "baptized" with Latin words describing the main property of the mutants, and the biochemical mutants according to the classic nomenclature. The wild type genes carry "+" or exponent; i.e., *z*⁺. Incompatibility genes are distributed among nine loci, each designated by a small letter. Bernet's symbols for the incompatibility genes are given in parenthesis.

Next to the number of linkage groups, on the straight line symbolizing the chromosomes, the centromeres are marked by dots. Under the heading "characteristics", only the main properties of the mutants are given as far as they are different from wild type properties: black spores, dark green-black mycelium with aerial hyphae and male and female sex organs. Since heterogenic incompatibility consists of two mechanisms, this has been marked.

Many strains which are mentioned in the table are kept in the collection in our laboratory. The spore mutants and some morphological mutants are kept in Prof. D. Marcou's laboratory (Laboratoire de Genetique, Faculte des Sciences, 91 Orsay, France). Most incompatibility strains are in the collection of Prof. J. Bernet (Laboratoire de Genetique, Faculte des Sciences, 351 Cours de la Liberation, 33-Talence, France).

Linkage group	Locus	Name	Number of alleles including wild type	Frequency of pat-meiotic reduction	Characteristics
	428		2	90	Spores green.
	485		2	85	Spores green.
	437		2	a3	Spores green.
	rib 1	riboflavin 1	2	70	Riboflavin-requiring; spores light green; mycelium sterile.
	k		2	12	
	rib 2	riboflavin 2	2	0	Riboflavin-requiring; spores dark green; mycelium sterile
	122		28	2	Spores colorless.
	f	flexuosa	5	81	Mycelium flat, no aerial hyphae.
	pa	pallida	2	97	Mycelium pale and sterile.
	+/-	mating type	2	98	

Linkage group	Locus	Name	Number of alleles including wild type	Frequency of post-meiotic reduction	Characteristics
II	52		2	24	Spores brown; mycelium pale.
	14	albospora=as	130	0.1-1	Spores colorless to dark green; mycelium colorless to wild type
	ci	circulosa	2	10	Clock mutant.
	p	pumila	2	19	Spores smaller; some spermatia sterile.
	385		2	78	Spores light green, persisting appendage.
	z	zonata	2	83	Mycelium brown; clock mutant; no ascogonia.
	457		2	83	Spores green.
III	477		2	89	Spores green.
	s		4	11	Heterogenic incompatibility, allelic mechanism from strain S.
	su-m		2	4	Suppressor for m (linkage group IV).
	b*	(Bernet: C)	16	4	Heterogenic incompatibility, non-allelic mechanism.
	t	(Bernet: B)	2	13	Heterogenic incompatibility, allelic mechanism.
	i	incoloris	36	80	Mycelium colorless, sterile.
	82		4	28	Spores yellow.
IV	a*	(Bernet: e)	16	18	Heterogenic incompatibility, non-allelic mechanism.
	m	minor	2	17	Smaller perithecia.
	49		2	15	Spores small, no germination.
	g	glaber	2	0.2	Mycelium smooth.
	su-1		2	20	Non-cistron-specific suppressor.
	oct	octospora	2	68	Irregular asci; clock mutant; nearly sterile.
	64		2	73	Spores have persistent appendix.
V	un	undulata	2	75	Clock mutant.
	v	(Bernet: R)	2	40	Heterogenic incompatibility, allelic and non-allelic mechanism.
	154		2	20	Spores have persistent appendix.
	sp	splendida	2	0.2	Mycelium glossy, sterile.
VI	la	lanosa	2	77	Mycelium velvety.
	lb	lano-alba	2	87	Mycelium velvety, white.
	ta	tarda	2	1.7	Slow growth; clock mutant.
	5		2	0.1	Spores green; mycelium pale.
VII	68		2	11	Spores small, no germination; mycelium sterile.
	110		3	16	Spores yellow; perithecia nearly sterile.
	63		2	30	Spores with persistent appendix, usually not germinating
	121		2	26 or 50?	Spores brownish.
	lg	lanuginosa	3	45	Mycelium velvety, sterile; slow growth.
	l	lenta	3	47	Mycelium velvety, sterile; very slow growth.
	ao	albo-lana	2	62	Mycelium velvety, white, sterile.
VIII	50		73	0-0.5	Spores dark green, of variable size.
	u	(Bernet: Q)	2	5	Heterogenic incompatibility, allelic mechanism.
	100		2	33	Spores yellow.
	401		2	66	Spores green.
	lp	lano-pallida	3	86	Mycelium velvety, white.
Genes not localized	al	alba	2	84	Mycelium bright.
	f'	fulva	2	83	Spores bright; mycelium brown.
	fl	fluctuosa	2	45	Clock mutant.
	c	(Bernet: P)	2	73	Heterogenic incompatibility, non-allelic mechanism.
	d*	(Bernet: D)		80	Heterogenic incompatibility, allelic and non-allelic mechanisms.
and more than 100 others (biochemical or morphological).					
*Many mutations have been isolated from some of the 16 wild type alleles of a, b and d loci.					

4. Genetic problems which have been or are being studied in *Podospora anserina*.

Besides the basic formal genetics initiated by Rizet in his laboratory, the following problems have been analysed:

Two phenomena of extrachromosomal inheritance: (a) the senescence syndrome, first instance of cytoplasmic inheritance described in a fungus (senescence, unavoidable by vegetative growth, is infectious for "young" cells) (Rizet, Marcou), and (b) the borroge phenomenon (the interaction of the two alleles S/s results in a cytoplasmically inherited gene inactivation) (Rizet, Beisson-Schecroun).

Physiology of rhythmic growth mutants (Nguyen van Huong).

Cytology of meiosis, with special reference to the kinetic apparatus (electron and light microscopy) (Zickler).

Mechanism of intragenic recombination, using spore color mutants (Marcou, Toure).

Identification of a polycistronic unit of transcription by complementation studies and use of non-sense suppressors (Marcou Picard).

Genetic control of incompatibility in 16 wild strains and physiology of the incompatibility in non-allelic systems, using a great number of mutations modifying or abolishing incompatibility (Bernet, Begueret, Belcour).

In our laboratory the following problems have been analysed or are under way. The names of the collaborators are given in parentheses

Heterogenic incompatibility (initiated by Rizet); the establishment of the basic genetic concept and first observations concerning the physiological mechanism. A biochemical analysis of this incompatibility system is now in progress (Blaich).

The cytology of ascospore formation, explaining the particular segregation pattern of alleles due to a regularly occurring specific spindle orientation (Franke).

Construction of chromosome maps, considering chromosome and chromatid interference; mapping functions (Kuenen).

Analysis of the genetic regulation of the formation of phenoloxidases (laccase and tyrosinase) per se and in connection with biochemical control of morphogenesis, using the numerous morphological mutants (Herzfeld, Molitoris, Minuth).

Biochemical analysis of the morphological mutants (Lysek).

Lost but not least, Perham (USA), in his Ph. D. thesis (1961 Florida State University), has studied the nutritional requirements and produced some auxotrophic mutants.

Since almost all of the original papers have been published either in French or in German, I would be glad to give a complete reference list in the next issue of the NN, if this should be desired.

5. Advantages of *Podospora anserina* as an object of genetic study.

The reader may ask, Why are these Europeans using such a strange and, from the first glance, rather complicated organism as *Podospora*, and, Why do they not integrate into the large family of Neurosporologists?

(1) *Podospora* has a short life cycle (E-15) days.

(2) As mentioned above, due to the relatively large size of the ascospores, they can be much more easily isolated than *Neurospora* spores.

(3) Spontaneous mutations occur very rarely. Mutations of all kinds (spore pigmentation, size or form, mycelial pigmentation, structure or growth, biochemical mutations, etc.) are inducible by different mutagenic agents: UV, X-rays, nitrosoguanidine, acridines (ICR-170), etc.

(4) Heterocaryons are formed by nature. Each postreduction event of a gene pair leads to a heterocaryotic spore. The resulting mycelia start growing with equal proportions of both nuclear components. This situation accomplishes directly the complementation test.

(5) There is no contamination within strains or to other strains, since there are no germinable conidia.

(6) The disadvantage of having no conidia for selective mutagenesis may be easily overcome by treating either small hyphal fragments obtained in a Waring blender or microconidia prior to crossing.

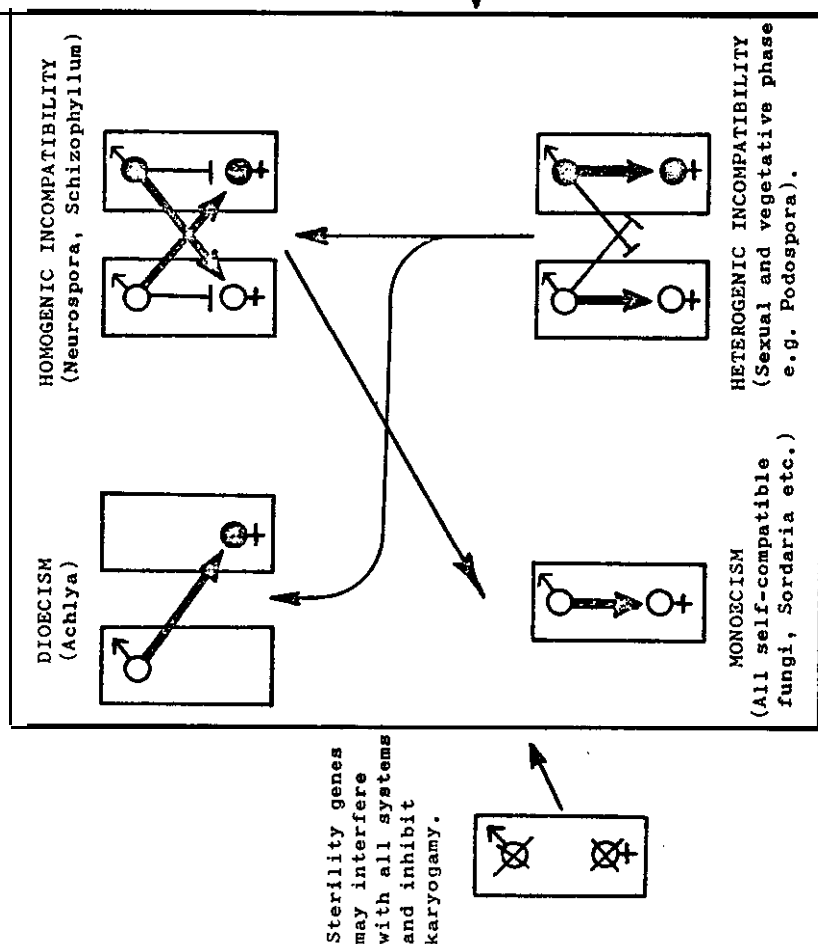
(7) The presence of uninucleate microconidia containing a very small amount of cytoplasm makes it possible to investigate nucleo-cytoplasmic relations. Furthermore, anastomoses between mycelial particles occur without nuclear migration.

(8) *Podospora* is particularly suitable for studying heterogenic incompatibility, since both cellular and sexual incompatibility are found, without formation of lethal progeny. Furthermore, just the confronting of two strains is sufficient to observe incompatibility: cellular incompatibility shows up as the "barrage" line and sexual incompatibility results in the absence of one or both lines of fruiting bodies corresponding to the two reciprocal crosses.

(9) Because of the pseudocompatibility, every wild race is isogenic. The existence of uninucleate spores of either + or - mating types makes, in fact, this organism heterothallic.

It is not the aim of this communication to run a band wagon for *Podospora*; but simply to call the attention of Neurosporologists to an organism which is closely related to *Neurospora*, not only from a viewpoint of taxonomy, but also as to what genetic problems are involved. If this presentation has the effect that some more research is done with this organism or that some *Podospora*ologists now working "underground" may show up, I would be very grateful.

SYSTEMS WHICH INCREASE OUTBREEDING



SYSTEMS WHICH DECREASE OUTBREEDING

Figure 1. BREEDING SYSTEMS IN FUNGI

The rectangles represent single individuals. The circles within the rectangles represent nuclei. Black and white colors indicate that these nuclei differ at least by one gene. If these nuclei are of different sex, the circles are replaced by the male and female sex symbols. A hermaphroditic character is represented by the inclusion of both sex symbols in the rectangle. If the symbols of the nuclei are crossed out, sterility is indicated by thick arrows. Compatibility is indicated by thin arrows. The T-shaped arrows indicate interactions between different systems.