Lineus as a model for studying developmental processes in animals reconstructed from adult pieces

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ABSTRACT The difficulties that prevent reconstruction of animals by piecing together body fragments from several adults are overcome by using nemerteans of the genus *Lineus*. For 25 years I have managed to make viable composite worms by grafting body parts cut out of *Lineus* specimens either from the same clonal strain (syngeneically reconstructed worms) or from the same species (allogeneically reconstructed worms) or else from different species (xenogeneically reconstructed worms). Body reconstruction has usually been carried out orthotopically, i.e., the components of composite animals have been selected so as to be anatomically complementary. However, reconstruction has been made heterotopically when it was essential to obtain morphogenetic events in the adults. Here, I shall review some of the developmental processes that took place in such reconstructed animals. First, I shall report immune responses in composite worms derived from various combinations of body pieces grafted together. Second, I shall study sex differentiation during gonad development in growing or regenerating chimeric worms made by the grafting of male and female components. I shall refer also to gonadogenesis in the asexual progeny of bipartite chimeras derived from lateral body halves of both sexes fused together (clones of bilaterally allophenic worms). Third, I shall analyze regulative processes (regeneration, transgeneration) during localized morphogenesis occurring in heterotopically reconstructed worms. The data show how reconstructed *Lineus* may be exploited to increase our knowledge of developmental mechanisms, especially in the misunderstood field of organismal pattern homeostasis.

KEY WORDS: *regeneration, transgeneration, sex differentiation, transplantation immunity, chimeras*

Introduction

The surgical difficulties as well as the biological impediments involved are still thought to prevent successful reconstruction of living animals by the piecing together of body fragments derived from several adults. However, during the last 25 years, I have managed to reconstruct a large number of viable organisms by grafting pieces from some or many nemertean specimens of the genus *Lineus* (Fig. 1). These animals produced by "zoosynthesis" (Bierne, 1970, 1979, 1980, 1985) thanks to an easy transplantation procedure (Bierne, 1970, 1985) have been manufactured from worm fragments that were either anatomically complementary (orthotopically reconstructed animals) or not (heterotopically reconstructed animals). The donors of worm pieces were chosen from among animals either of the same clonal stain produced by asexual multiplication (intraclonal reconstruction = syngeneically reconstructed worms) or of the same species (intraspecific reconstruction = allogeneically reconstructed worms) or else of different species (interspecific reconstruction= xenogeneically reconstructed worms).

In the present paper I shall review some of the developmental responses that resulted from such animal reconstructions. Immunogenesis is studied in composite worms derived from various combinations of body pieces fused together so that the area producing antigraft immunocytes can be localized in the intestinal region of nemerteans. Sex differentiation of gonads is studied in regenerating heterosexual chimeras made from male and female components so that the relative proportion of male- and female-determining cells in the gonad can result in the sexual phenotype. Regulative processes are analyzed during local morphogenesis occurring in heterotopically reconstructed worms so that duality of responses (regeneration or transgeneration) to morphological abnormalities can support the concept of organismal pattern homeostasis.

Abbreviations used in this paper: MST, median survival time.

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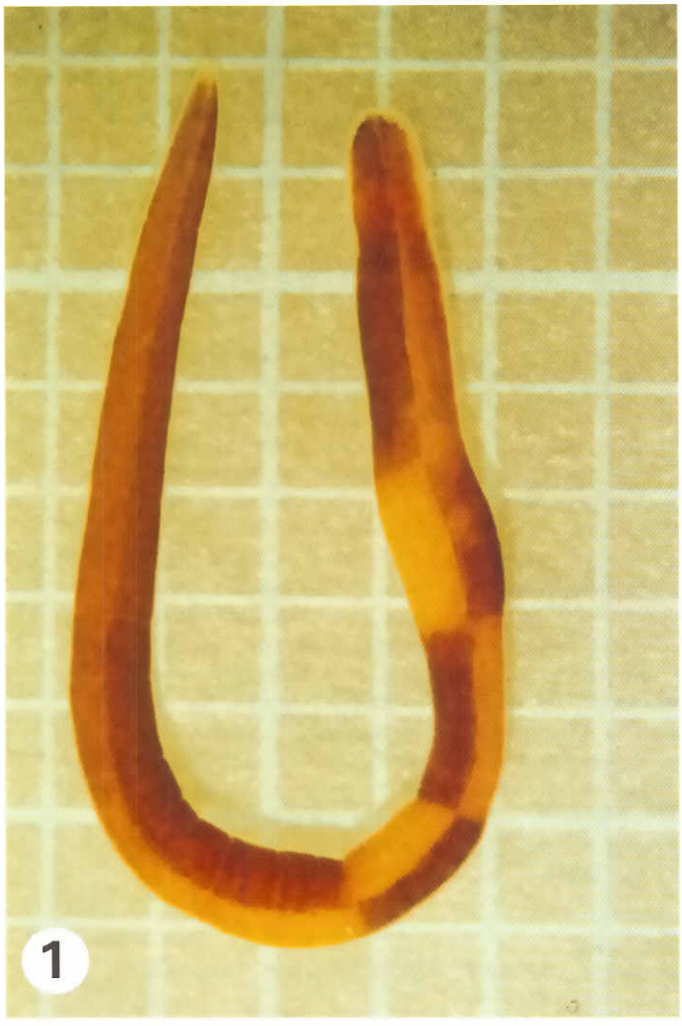


Fig. 1. A multiparental *Lineus* chimera. *This worm reconstructed by piecing together 10 anatomically complementary fragments from 8 different adult* Lineus ruber *is the first hexadecaparental animal in the world. The patchwork design of this composite nemertean was obtained by grafting pieces of alternating contrasting skin colors. The bilaterally two-colored posterior end of the multiparental chimera was initially no longer than the other bilaterally two-colored body parts but its length increased about four-fold from the 4th to the 11th month after piecing. The scale is indicated by the background which is marked off in millimeters.*

Immunogenesis in composite worms derived from various combinations of body pieces grafted together

For a long time, cells responsible for the specific immune response to foreign grafts have been thought to exist only in vertebrates. However, extensive studies of interspecific graft behavior in nemertean worms of the genus *Lineus* provide strong indications that these marine invertebrates also possess such cells (Langlet and Bierne, 1982a). To learn more about these putative immunocytes, Langlet and I have studied the survival of transplants from donors of one *Lineus* species grafted onto recipients previously reconstructed from two other *Lineus* species (Langlet and

TABLE 1

MSTs OF ANTECEREBRAL ENDS GRAFTED ONTO MONOSPECIFIC CONTROLS

Donors of grafts	Recipients	No and (%) of original grafts surviving at days after transplantation										MST (confidence limits)
		1	5	10	15	20	30	40	50	60	90	
Ls	Ll	20 (100)	20	20	20	20	20	20	20	20	20 (95)	>90
Ls	Lr	18 (100)	18	18	13 (72)	1 (7)	0 (0)	-	-	-	-	15.4 (14.7-16.1)
Lr	Ll	15 (100)	15	12 (80)	12	12	12	11 (73)	6 (40)	4 (26)	4	45
Lr	Ls	25 (100)	25	22 (88)	15 (60)	9 (36)	0 (0)	-	-	-	-	16.4 (14.1-19)

Ll: Lineus lacteus; Lr: Lineus ruber; Ls: Lineus sanguineus

TABLE 2

MSTs OF ANTECEREBRAL ENDS GRAFTED ONTO BISPECIFIC CHIMERIC RECIPIENTS

Donors of grafts	Chimeric recipients components	join	No and (%) of original grafts surviving at days after transplantation 1	5	10	15	20	30	40	50	60	90	MST (confidence limits)
Ls	Lr→Ll	p.eso	14 (100)	14	14	14	14	14	14	13 (92)	13	13	>90
Lr	Ls→Ll	p.int.	19 (100)	19	19	19	15 (79)	12 (63)	6 (31)	5 (26)	4 (21)	2 (10)	36
		int.	8 (100)	8	5 (62)	1 (12)	0 (0)	-	-	-	-	-	10.6 (8.9-12.5)
Lr	Ll→Ls	p.int.	6 (100)	6	5 (83)	0 (0)	-	-	-	-	-	-	11 (10.1-11.8)
		int.	5 (100)	3 (60)	1 (20)	1	0 (0)	-	-	-	-	-	6.4 (4.1-9.9)

Ll: Lineus lacteus; Lr: Lineus ruber; Ls: Lineus sanguineus; p.eso: preesophageal; p.int: preintestinal; int.: intestinal

Bierne, 1979, 1982b, 1983, 1984). Three "graft-bispecific recipient" combinations have been tested. First, antecerebral ends from *L. ruber* donors were grafted onto chimeric worms previously constructed from *a L. sanguineus* anterior component and *a L. lacteus* posterior component. Second, similar grafts (antecerebral ends from *L. ruber*) were transplanted onto bispecific chimeras constructed from a *L. lacteus* anterior component and a *L. sanguineus* posterior component. Third, antecerebral ends from *L. sanguineus* donors were grafted onto chimeric worms previously constructed from a *L. ruber* anterior component and a *L. lacteus* posterior component. When the intestinal segment of the chimeras consisted exclusively of *L. lacteus* tissues, the median survival times of the grafts were in accordance with those characteristic of the interspecific "graft donor - *L. lacteus*" combination. On the other hand, when at least part of the intestinal segment was from *L. sanguineus*, the median survival times of the grafts were in accordance with those characteristic of the "graft donor - *L. sanguineus*" combination (Tables 1 and 2).

These findings showed clearly that the survival of incompatible xenogeneic grafts in *Lineus* chimeras is dependent only on the compatibility of the antecerebral graft cells and those from the intestinal segment of the recipient's body. Thus, the immunocompetent anatomical region of *Lineus* can be located in the intestinal segment, where the cells that are responsible for the rejection of the distant antecerebral graft are generated. These immunocompetent cells are very likely mobile nemertine blood cells, with recognition and cytotoxic functions.

To support the hypothesis that the *Lineus* intestinal region is responsible for the production of antigraft immunocytes, C. Langlet, D. Brossard and I have recently tested the survival of incompatible

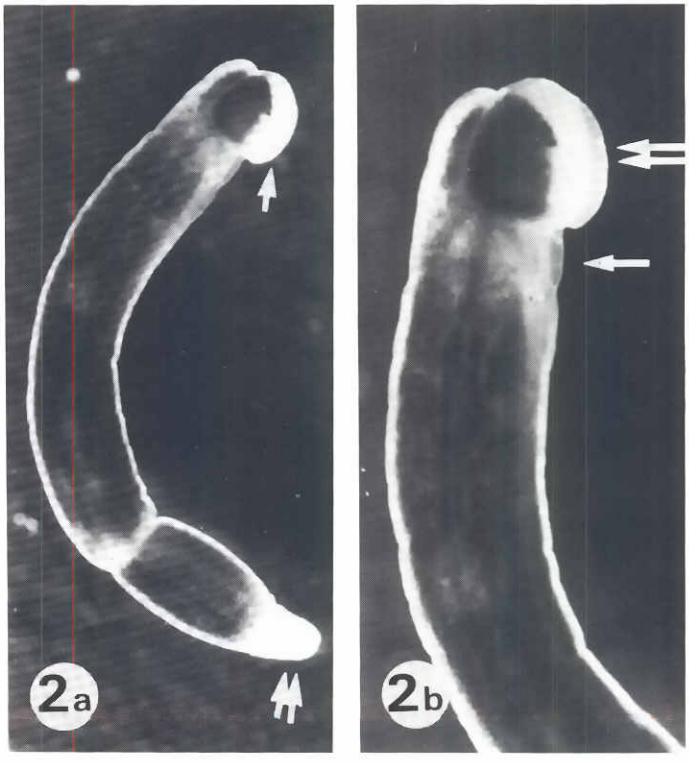


Fig. 2. *L. sanguineus* reconstructed by piecing together preintestinal regions from specimens of the same clonal strain. (a) *The resulting specimen does not reject the antecerebral half from* L. ruber *(arrow) 50 days after grafting. The double arrow shows a posterior regenerate before amputation. x15.* **(b)** *Detail of the head in* **(a)** *showing that the graft cephalic slit (double arrow) is unaffected and in perfect continuity with that (arrow) of the "enterectomized" recipient. x30.*

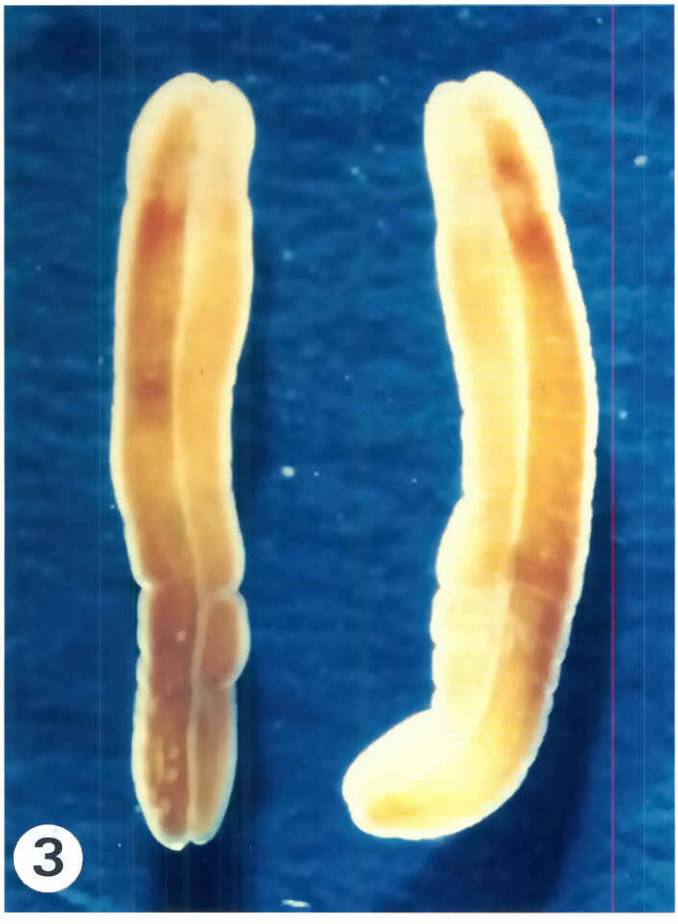


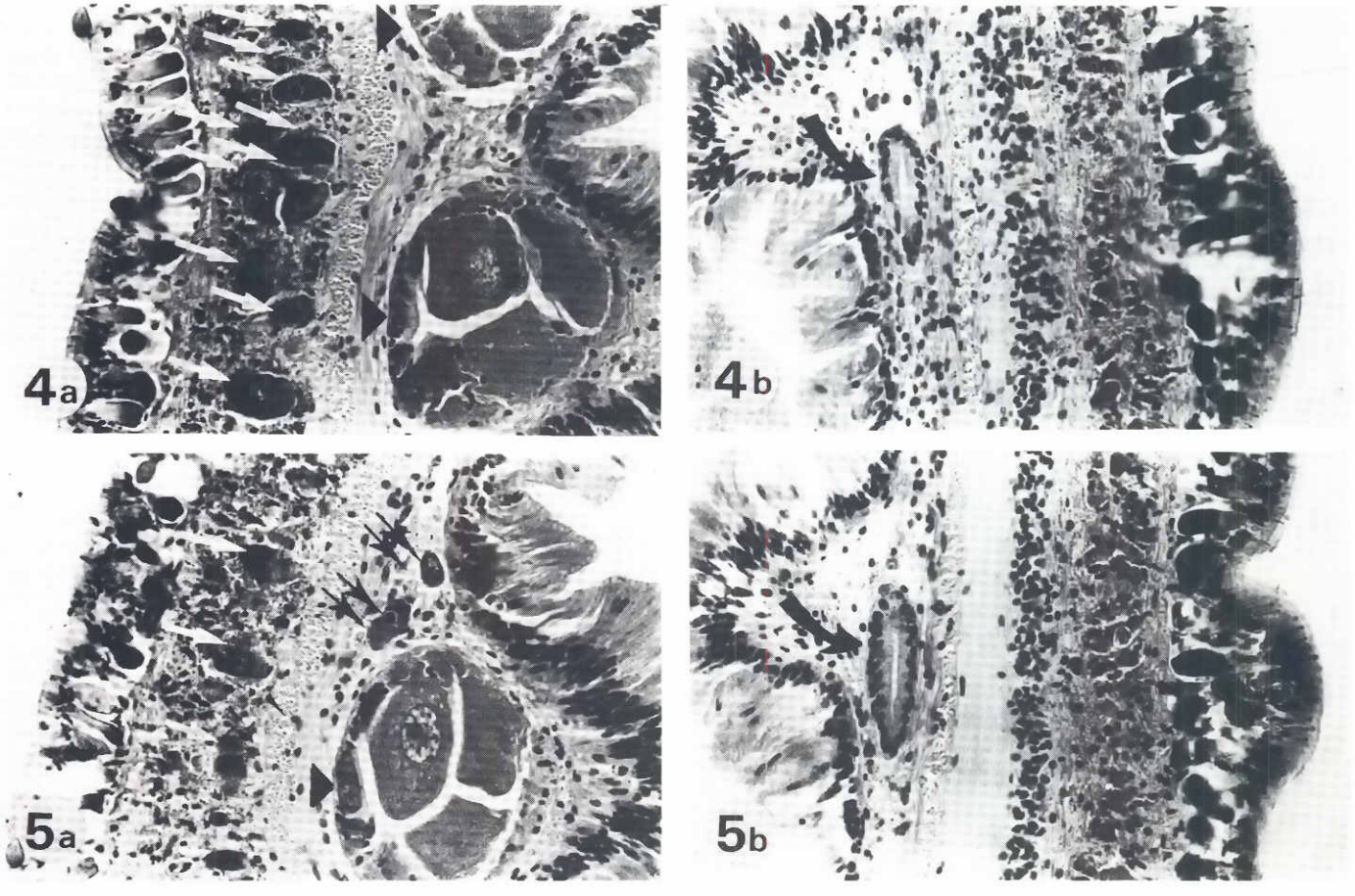
Fig. 3. Two ♂↔♀ chimeras made by grafting complementary lateral halves from male and female *L. sanguineus* *in a reciprocal arrangement (after one month's reconstruction). x20.*

grafts in animals reconstructed without the intestine (Bierne *et al.*, 1989a). Such intestine-deprived worms of adequate size, i.e., comparable to controls, were obtained by piecing together a number of preintestinal regions (previously decapitated except the most anterior segment) from *L. sanguineus* specimens of a clonal strain. Three months later, the left and right antecerebral end halves from donors of another *Lineus* species, *L. ruber*, were grafted respectively onto controls from the clonal strain and worms reconstructed without the intestine. Then, the posterior ends of controls and intestine-deprived worms were amputated weekly to prevent the regeneration of a functional intestine in the reconstructed worms. Control worms rejected xenografts according to the pattern indicated in previous studies (Median survival time (MST) of grafts = 16 days). On the other hand, the intestine-deprived worms did not (Graft MST > 100 days). Since "enterotectomized" nemerteans fail to reject incompatible grafts (Fig. 2) as neonatally thymectomized mice do, we propose the term "thymus-like" for the nemertean intestine. In the marine *L. sanguineus*, antigraft immunocytes are probably intestine-dependent immunocytes (I-cells), i.e., endoderm-derived organ-dependent cells, as are vertebrate T-cells.

Sex differentiation of gonads in growing or regenerating male-female chimeric *Lineus* and their possible sexual progeny

All species of the nemertine genus *Lineus* are strictly gonochoristic animals. For 25 years I have constructed many intra- (allo) or interspecific (xenogeneic), heterosexual chimeras of various ♂↔♀ designs (bipartite, anterior-posterior, sandwich, patchwork, etc.). For the investigation of sex differentiation in these heterosexually reconstructed worms I used two strategies: either to cause gonad regeneration from castrated worms or to wait for gonad development to occur cyclically (annually in nemerteans) after each resting period of reproduction (Bierne 1970, 1975; Vernet and Bierne, 1988). From species that can be propagated asexually such as *L. sanguineus*, bipartite ♂↔♀ chimeras (Fig. 3) could be divided transversally into many pieces to produce clones of bilaterally allophenic animals by worm regeneration from fragments (Sivaradjam and Bierne, 1980, 1981, 1985, 1989).

In allogeneic chimeras as well as in allophenic worms sex reversal of the gonads occurred either in the male or in female components close to the components of the opposite sex according to a species-specific pattern (masculinization of ovaries in ♂↔♀ *L. ruber* chimeras; feminization of testes in ♂↔♀ *L. sanguineus* chimeras and their asexual progeny, the bilaterally composite worms). However, the stray germ cells in the connective tissue around normal or sex-reversed gonads of components of both sexes always entered meiosis and differentiated oocytes. From recent observations in xenogeneic ♂/ *L. ruber*↔♀ *L. viridis* chimeras (Bierne *et al.*, 1989b), abnormal migration of germ cells was recorded in the ♀ *L. viridis* components. These ectopic germ cells located in the skin entered meiosis, differentiated oocytes and



Figs. 4 and 5: Sections in the *L. viridis* component of an anterior-posterior ♂ *L. ruber* ↔ ♀ *L. viridis* chimera. (4a and 5a) *Ectopic oocytes and stray oocytes respectively located in the skin (arrows) and in the connective tissue (double arrows) of the left body half whose ovaries are fertile (arrowheads).* **(4b and 5b)** *Sterile gonads (arrows) in the right body half whose skin is normal, without ectopic germ cells. x500.*

grew to a considerable size, i.e., 30 µm in diameter (Figs. 4a and 5a). Closely connected with the aberrant location of oocytes in a lateral body half whose ovaries were fertile, sterility of contralateral gonads occurred (Figs. 4b and 5b). Germ cells outside gonads never differentiated along a male line. From these findings two questions on the sex differentiation in *Lineus* arise: 1) Why do germ cells in testes not differentiate oocytes? 2) How can testes or ovaries in ♂↔♀ composite worms produce an aberrant gametogenesis? For the first question, a possible answer may be the presence of a putative meiosis-inhibiting substance (MIS) secreted only by testes whereas in the absence of this MIS, germs-cells in ectopic location as well as in ovaries enter meiosis and start up oogenesis. For the second question, the secretion of MIS, by sex-reversed ovaries and the inhibition of MIS secretion by sex-reversed testes may then be considered. Whether the gonadal sex-reversal results from cell migling or diffusible substances from male and female chimera components is not yet known. However, grafting manipulations with body fragments from completely feminized allophenic nemertines obtained by vegetative multiplication of bipartite ♂↔♀ chimeras showed their ability to reexpress the "testis" characteristic (Sivaradjam and Bierne, 1987). Such experiments on the sex-reversed reversal suggest that the relative proportion of male- and female-determining somatic cells in the gonads might decide their sexual phenotypes. To prove that this relative proportion results in the gonadal sex as it probably does in mammals (Mc Laren, 1984) is a challenge for future research.

Regulative morphogeneses occurring in heterotopically reconstructed *Lineus*

When an animal is heterotopically reconstructed, discontinuities in the sequence of positional information values assigned to cells (Wolpert, 1969) occur along one or more axes (anterior-posterior, dorsal-ventral, medial-lateral) or the three-dimensional system of body pattern coordinates. Two questions arise: whether or not a developmental event takes place in response to a disruption introduced into the series of positional values of the reconstructed organism's cells; and if so, what kind of morphogenetic response occurs.

I now come to the developmental responses to disruptions inserted in the series of positional values along the anterior-posterior axis in *Lineus*. These responses were obtained by mani-

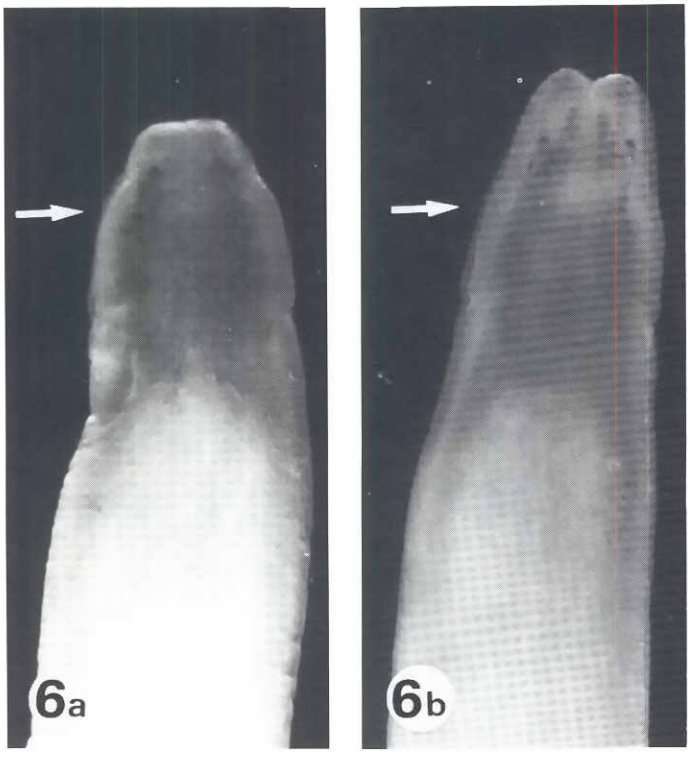


Fig. 6. Intercalary regeneration occurring after the introduction of a shortage of positional values in the antecerebral end of a *L. pseudolacteus* worm. *The arrow indicates the anterior end of the cerebral ganglia. Photographs at 7* **(a)** *and 30* **(b)** *days postgrafting. x50.*

pulating the pattern of the anterior cephalic region — the antecerebral end — in a large number of specimens of several *Lineus* species *(L. ruber, L. viridis, L. sanguineus, L. lacteus, L. pseudolacteus)*. This is the only worm compartment in which no nerve cell bodies are found. Thus, it is a suitable experimental system for making comparisons with results from experiments concerning morphogenetic behavior in abnormally reconstructed amphibian limbs or insect legs, which also lack nerve cell bodies (Bierne, 1988; Bierne and Jean, 1989; Jean and Bierne 1989; Bierne *et al.*, 1989c).

When discontinuities in the sequence of positional values were due to a shortage of these values, complete resolution of abnormalities occurred in all cases by intercalary regeneration (Figs. 6, 8 and 9). Pigmentary differences between the graft (from a pale or dark species) and the recipient (from a dark or pale species) showed that the intercalary regenerate resulted from both, i.e., from tissues anterior and posterior to the intercalate (Figs. 8 and 9). These findings indicate that regeneration occurs by a cell round-trip mechanism, and not by a simple addition of cells derived from either the recipient (posterior component of the heterotopically composite animal) or the graft (anterior component of the heterotopically composite animal). In adult metazoans, such a morphogenetic response is dependent on their regenerative capacities. Thus, it has been shown that an intercalation (i.e. a localized regenerative growth that restores the original pattern) takes place in amphibian limbs and in insect legs after a deletion along the proximodistal axis (Bryant *et al.*, 1977).

On the other hand, when disruptions in the sequence of positional values were derived from an excess of these values, pattern discontinuities were resolved by a regressive process in all cases (Figs. 7 and 10). I have created the term "transgeneration" to describe the morphogenetic regulation that takes place through decrease in cell number in body parts duplicated by heterotopic transplantation. In adult animals capable of regeneration, such a developmental process, occurring in specimens experimentally given an excess of body regions, has not been reported up to now. In previously described cases, a duplication of positional values resulted either in maintenance of the abnormality or in supernumerary, intercalary regeneration. For example, surplus was maintained in amphibian limbs characterized by a duplication along the proximal-distal axis. On the other hand, intercalation adding more material to the excess already present occurred in similarly reconstructed insect legs (Bryant *et al.*, 1977).

The present data give evidence for a dynamic stability of patterns in adult *Lineus* since a morphogenetic event can occur in response to the experimental introduction of a disruption in the series of positional value. When cells are no longer surrounded by their usual neighbors, they change course and the abnormality changes into the normal form.

It is known that this homeostasis of patterns is shared by metazoans capable of regeneration by epimorphosis (reprogrammed growth) as well as by morphallaxis (*in situ* reprogramming without growth). However, an animal regenerates either by epimorphosis or

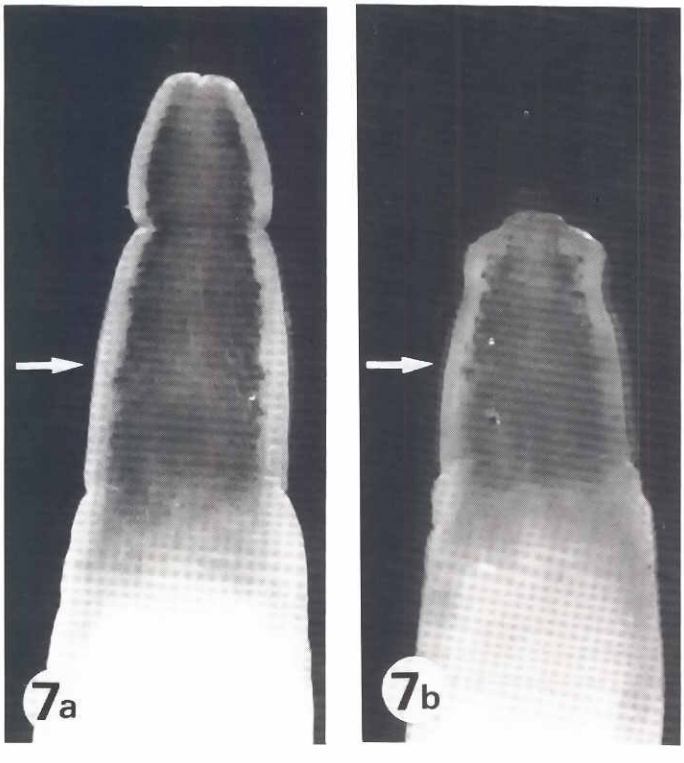
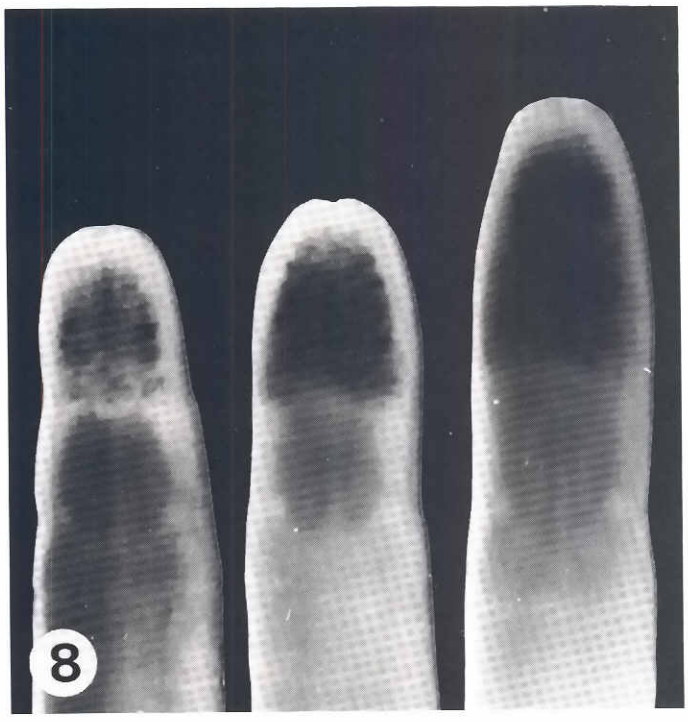
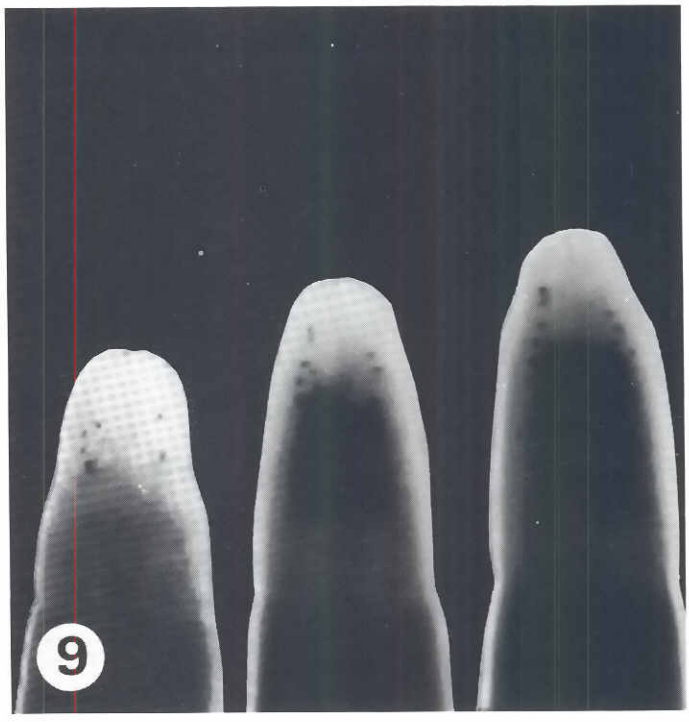


Fig. 7. Intercalary transgeneration occurring after the introduction of an excess of positional values in the antecerebral and of a *L. pseudolacteus* worm. *The arrow indicates the anterior end of the cerebral ganglia. Photographs at 7* **(a)** *and 60* **(b)** *days postgrafting. x50.*





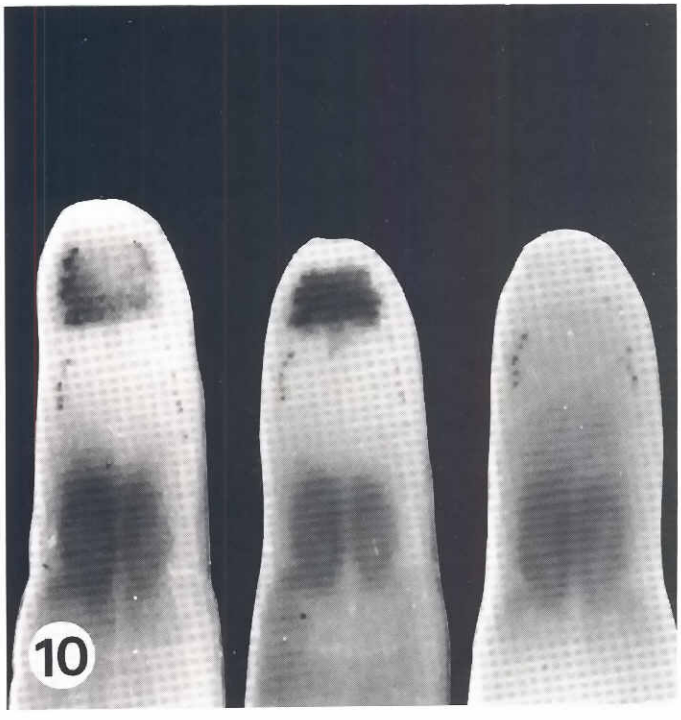


Fig. 8. Intercalary regeneration occurring after the introduction of a shortage of positional values in the antecerebral end between a *L. sanguineus* graft and a *L. lacteus* recipient. *The intercalary regenerate is pigmented like the graft. Photographs at 4 (left), 12 (center) and 24 (right) weeks postgrafting. x30.*

Fig. 9. Intercalary regeneration occurring after the introduction of a shortage of positional values in the antecerebral end between a *L. lacteus* graft and a *L. sanguineus* recipient. *The intercalary regenerate is pigmented like the recipient. Photographs at 4 (left), 12 (center) and 24 (right) weeks postgrafting. x30.*

Fig. 10. Intercalary transgeneration occurring after the introduction of an excess of positional values in the antecerebral end between a *L. sanguineus* graft and a *L. lacteus* recipient. *Photographs at 4 (left), 12 (center) and 24 (right) weeks postgrafting. x30.*

by morphallaxis, but the two processes never occur alternately. Moreover, both epimorphic and morphallatic regeneration are developmental processes which follow an amputation resulting in a deletion of positional values. The distinct morphogenetic response of heterotopically reconstructed *Lineus* is the morphallatic response to an excess resulting from a duplication of positional values. This morphallaxis does not convert one body region into another but reduces excess material inserted between two body regions. This important difference justifies the term transgeneration which describes the resolution of an abnormal pattern by selective regression.

The rule of intercalary regeneration is well known: discontinuities in the sequence of positional values provoke local growth and the newly grown cells take on intermediate positional information

values that restore continuity in the pattern. The rule of transgeneration can be specified by the developmental rule of diminution: discontinuities in the sequence of positional values provoke local regression and the remaining cells take on intermediate positional values that restore continuity in the pattern. Further work will attempt to answer the question: why does the morphogenetic response from *Lineus* to a discontinuity in the pattern of positional values depend on the polarity of the sequence of cells which are opposite each other (cephalic-caudal versus caudal-cephalic)? Furthermore, the questions raised by Bryant and Muneoka (1986) as to "how the positional information is encoded within the cell and how it is used during development" will also require further consideration.

The transfer and processing of positional information during regeneration in *Lineus* reconstructed with a surplus have provided information about the morphogenetic code. M.F. Desselle and I have constructed allogeneic and xenogeneic chimeras by grafting the anterior parts from specimens of *L. sanguineus* transected at the intestinal level onto *L. sanguineus* or *L. lacteus* worms previously decapitated at the esophageal level. One month later, the heterotopically reconstructed worms were caused to regenerate by transection, keeping a "slice" of the intestinal region of the anterior component (surplus) in front of the esophageal region of the posterior component (recipient).

Anatomical and genetic markers in the two components helped to elucidate the respective roles of surplus and recipient in the regenerative morphogenesis (Deselle and Bierne 1980, 1982 and in press). By staying within structures of unchanging morphology, anatomy and pigmentary phenotype, and by generating no offspring in the blastema, the recipient cells showed that they do not take part in the field of blastocyte recruitment. On the other hand, the regulations that occurred during regeneration from thin "slices" (thickness <500 μm) proved that the recipient can control cell positioning in the regenerate. By comparing the way in which aberrant patterns reimpose themselves when the "slices" were thick (> 500 μm), the pattern regulations allow us to define a positioning field whose area in the body is just sufficient to initiate a corrective + palliative morphogenesis. In other words, regulation is superposed to regeneration when the boundaries of the positioning and recruitment fields are on both sides of the discontinuity inserted by reconstruction.

Since the gap between the two fields controls the extent of regulation, it is suggested that the mechanism of corrective morphogenesis is inhibitory. Signals derived from cells of the positioning fields are probably negative instructions, i.e., information that stops the progress of the morphogenesis.

Concluding remarks

A few classes of metazoans have provided experimental biologists with a genus-model chosen for its unusual properties and/or its singular features. I think that *Lineus* serves well as a biological model because of its clonability and its reconstructability. Many developmental processes can be investigated in adult worms such as immunogenesis, sex differentiation, morphological homeostasis, etc. Moreover, *Lineus* could become an artistic toy since it allows us to use living tissue as a material for construction games and sculpture.

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References

BIERNE, J. (1970). Recherches sur la différenciation sexuelle au cours de l'ontogenèse et de la régénération chez le Némertien *Lineus ruber Müller*. *Ann. Sci. Nat. Zool. 12*: 181-298.

BIERNE, J. (1975). Sex differentiation in regenerating ♂/♀ nemertine chimeras. In *Intersexuality in the Animal Kingdom* (Ed. R. Reinboth). Springer Verlag, Berlin, Heidelberg, New York, pp. 30-40.

BIERNE, J. (1979). Reconstitution d'animaux viables par la greffe de nombreux morceaux prélevés sur plusieurs *Lineus* adultes. *Arch. Anat. Microsc. 68*: 219.

BIERNE, J (1980). Viable animals obtained by grafting pieces from several nemertean adults. *Transplantation 29*: 74-76.

BIERNE, J. (1985). Histocompatibility in nemertines: fates of multiparental *Lineus* constructed by grafting of pieces from many donors. *Am. Zool. 125*: 135-144.

BIERNE, J. (1988). Transgeneration as an alternative to regeneration in heterotopically reconstructed *Lineus*. In *Control of Cell Proliferation and Differentiation during Regeneration* (Ed. H.J. Anton). S. Karger, Basle, pp. 230-234.

BIERNE, J., BROSSARD, D. and FARGETTE-LANGLET, C. (1989a). Thymus-like intestine in the marine nemertean *Lineus sanguineus*. *Cell Differ. Dev. 27* (Supp.): 198.

BIERNE, J., CARLIER, A. and RUE, G. (1989b). Germ cells outside gonads of ♂ ↔♀ nemertine chimeras enter meiosis and differentiate oocytes. *Cell Differ. Dev. 27(Supp.)*: 492.

BIERNE, J., JEAN, M. and RUE, G. (1989c). Information de position et contrôle de la prolifération cellulaire *in vivo*. *Reprod. Nutr. Dev. 29* (2) (Supp.): 7.

BIERNE, J. and JEAN, M. (1989). Insertion of an excess in the sequence of AP positional information values of the antecerebral end cells can inhibit regeneration in *Lineus*. *Biol. Struct. Morphogen. 2*: 41.

BRYANT, P.J., BRYANT, S.V. and FRENCH, V. (1977). Biological regeneration and pattern formation. *Sci. Am. 237*: 66-81.

BRYANT, S.V. and MUNEOKA, K. (1986). View of limb development and regeneration. *Trends Genet. 2*: 153-158.

DESSELLE, M.F. and BIERNE, J. (1980). Transdifférenciation de greffons hétérotopiques chez des Némertes du genre *Lineus* en voie de régénération. *C.R. Séances Acad. Sci. (Paris) 291*: 837-840.

DESSELLE, M.F. and BIERNE, J. (1982). Marqueurs allogéniques et xénogéniques de la transdifférenciation de greffons hétérotopiques chez les Némertes du genre *Lineus* en voie de régénération. *Arch. Anat. Microsc. 71*: 294.

DESSELLE, M.F. and BIERNE, J. (1990). Transfert et traitement de l'information de position au cours de la régénération des Némertiens du genre *Lineus*. *Ann. Sci. Nat. Zool.* (In press)

JEAN, M. and BIERNE, J. (1989). Kinetics of morphogenetic responses from *Lineus* to discontinuities inserted in the sequence of AP positional information values of the antecerebral end cells. *Biol. Struct. Morphogen. 2*: 41.

LANGLET, C.L. and BIERNE, J. (1979). Intervention d'immunocytes circulants dans le rejet des greffes chez les Némertiens du genre *Lineus*. *C.R. Séances Acad. Sci. (Paris) 288*: 1003-1006.

LANGLET, C.L. and BIERNE, J. (1982a). Immune characteristics of graft rejection in nemerteans of the genus *Lineus*. *Eur. J. Immunol. 12*: 705-708.

LANGLET, C.L. and BIERNE, J. (1982B). Immunocompetent cells are responsible for rejection of incompatible xenogeneic grafts in *Lineus* (Invertebrata, Nemertea). *Transplantation 34*: 8-12.

LANGLET, C.L. and BIERNE, J. (1983). Experimental evidence for cell-mediated immune responses to incompatible grafts in *Lineus*. *Dev. Comp. Immunol. 7*: 617-620.

LANGLET, C.L. and BIERNE, J. (1984). Immunocompetent cells requisite for graft rejection in *Lineus* (Invertebrata, Nemertea). *Dev. Comp. Immunol. 8*: 547-557.

McLAREN, A. (1984). Chimeras and sexual differentiation. In *Chimeras in Developmental Biology* (Eds. N. Le Douarin and A. McLaren). Academic Press, London, pp. 381-399.

SIVARADJAM, S. and BIERNE, J. (1980). Féminisation des Némertes allophéniques produites par multiplication végétative de deux chimères bipartites. *C.R. Séances Acad. Sci. (Paris) 291*: 993-996.

SIVARADJAM, S. and BIERNE, J. (1981). Sex differentiation in bilaterally allophenic animals produced by cloning of two bipartite male/female chimeras of *Lineus sanguineus*. *J. Embryol. Exp. Morphol. 65*: 173-184.

SIVARADJAM, S. and BIERNE, J. (1985). Inversion complète du sexe de la composante mâle des Némertes allophéniques de deux clones produits par multiplication végétative de chimères hétérosexuées. *C.R. Séances Acad. Sci. (Paris) 301*: 335-338.

SIVARADJAM, S. and BIERNE, J. (1987). Réexpression du caractère "testicule" chez des Vers reconstruits à partir de composants de Némertes allophéniques totalement féminisées. *Arch. Anat. Microsc. 75*: 197.

SIVARADJAM, S. and BIERNE, J. (1989). Transmission et expression des caractères "ovaire" et "testicule" dans des clones de Némertes allophéniques produits par multiplication végétative de chimères bipartites mâle/femelle et femelle/mâle chez *Lineus sanguineus*. *Invertebr. Reprod. Dev. 16*: 103-110.

VERNET, G. and BIERNE, J. (1988). Neuroendocrine control of gonadogenesis in regenerating *Lineus lacteus* (Heteronemertea). *Hydrobiologia 156*: 53-60.

WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol. 25*: 1-47.