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Life History of *Coelomomyces psorophorae*

(mosquito pathogen/*Cyclops*/sexuality/alternate hosts/alternation of generations)

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ABSTRACT **The mosquito parasite, *Coelomomyces psorophorae* (Blastocladiales, Chytridiomycetes) alternates obligately between the larvae of *Culiseta inornata* and the copepod *Cyclops vernalis*. Isogametes, derived from heterothallic, wall-less gametangia which develop in the copepod, fuse to produce a diploid zygote that subsequently infects the mosquito host. Zoospores from the resistant sporangia which are produced in the haemocoel of the mosquito infect the copepod. A tentative life-history is proposed and implications of these discoveries for the biology, taxonomy, and possible role of *Coelomomyces* in biological control are discussed.**

The water mold *Coelomomyces* (Chytridiomycetes) is a host-specific, obligate parasite of the larvae of mosquitoes, black flies, chironomids, and tabanids (1–5). The fungus develops in the haemocoel of the host as a weakly-branched coenocytic thallus. At maturity, this vegetative structure differentiates into a number of thick-walled resistant sporangia (RS) (6). Under appropriate conditions, each of these sporangia will give rise to several hundred posteriorly uniflagellate zoospores. The genus includes pathogens of the major genera of mosquitoes and may cause significant epizootics in mosquito populations. Preliminary field trials have suggested that *Coelomomyces* may have potential as a biological control agent of mosquitoes (7–11). Efforts to explore this possibility have encountered significant difficulties when workers attempted to establish infected colonies in the laboratory. Larvae bathed in high densities of zoospores from the RS did not become infected. Couch (12) has developed a technique for mass production of infected larvae but the conditions leading to infection are complex and include a number of living components other than the host and its parasite.

Our studies have utilized *C. psorophorae* in *Culiseta inornata*. This species of *Coelomomyces* is a common parasite of mosquitoes in the irrigated regions of southern Alberta (10). Establishment of an infected colony in the laboratory was achieved by use of the Couch procedure (12) along with additions of various components of the biota from the original habitat, e.g., microalgae, arthropods, etc. Attempts to standardize infection rates by controlling the environment, density of the RS-zoospores, or age and condition of the host were unsuccessful. Attempts to reduce the biotic complexity of our gross cultures typically resulted in loss of infection. The source of this problem was revealed when single components of the complex or "gross" system were added individually to "clean" pans containing only the fungus and host insects. When the copepod *Cyclops vernalis* was added to pans containing RS and larvae, infection was obtained. Control pans lacking this crustacean consistently failed to yield infected mosquitoes. These results suggested that *Cyclops* might be serving as an alternate host for *Coelomomyces* (13). This paper presents evidence confirming this hypothesis and outlines a previously unknown life history.

Abbreviation: RS, resistant sporangia.

MATERIALS AND METHODS

A colony of *Culiseta inornata* was developed from egg rafts collected from infection sites near Lethbridge and Fincastle, Alberta, Canada. Rearing techniques were based on those of McLintock (14). The water used for larval rearing and infection trials contained 0.5 g of $NaHCO_3$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of KCl, and 0.5 g of $Ca(NO_3)_2 \cdot 4H_2O$ per liter of distilled water, with a final pH of 8.2. Larvae were fed a mixture of 60% enriched flour, 25% nonfat dried milk, 10% yeast extract, and 5% liver powder as required for optimal growth.

Resistant sporangia (RS) from 10 dead larvae, either from field or laboratory infections, were washed in distilled water, collected on NC Millipore filters, placed in individual moist chambers, and stored at 5°. When needed, sporangia were transferred to a dilute salt solution (15) and held at 20° under continuous illumination [with cool-white fluorescent light at 65 foot candles (699.7 lx)]. After 5 days, 80% of the sporangia were ready to release zoospores.

Infection trials were typically carried out in plastic pans (27 cm in diameter) containing 1.8 liters of water and held at 20 ± 2° in diffuse light. Bottom material (75 cm^3) from the colony rearing trays was autoclaved and added to each pan. Most mosquito infection trials were initiated with 125 late second-instar larvae and RS from one filter. Larvae were fed 0.2 g of food every other day. Addition of the food also initiates zoospore release by lowering the oxygen tension.

A population of *Cyclops vernalis* from the main infection site near Fincastle, Alberta, has been maintained along with the mosquito larvae. Infected copepods were reared in 37- by 48-cm pans containing the standard water and bottom material and received about two RS-filters per week.

Material was prepared for electron microscopy by fixation with glutaraldehyde and post-fixation in osmic acid (16, 17).

RESULTS

The first indication that copepods might be involved in the *Coelomomyces* infection problem came from pans which contained *Cyclops* as well as RS and mosquitoes (Table 1,A). If these copepods were serving as an alternate host for *Coelomomyces*, then one might expect that animals from pans with a history of high infectivity should be able to transmit the disease without the addition of other fungal inocula. This presumption was supported when carefully washed copepods from infective pans were added to pans containing mosquito larvae but lacking RS and RS-zoospores (Table 1,B). A direct

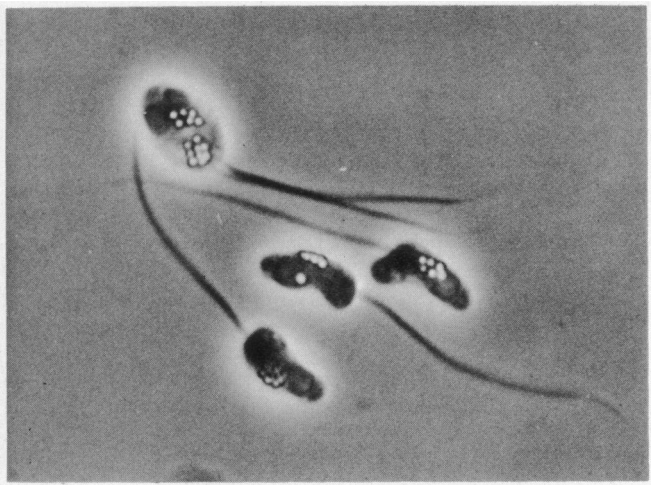

FIG. 1. Three isogametes and one zygote (top) of *C. psorophorae*. The recently fused zygote with two flagella still display-two adjacent nuclear caps and two side bodies (groups of refractile lipid granules). ×2070.

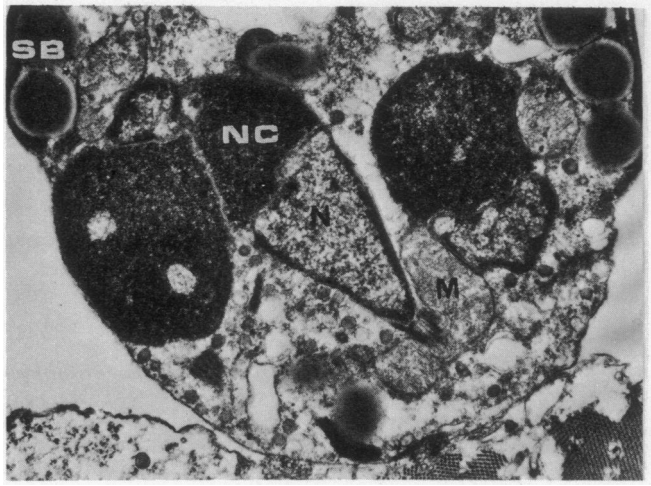

FIG. 2. Section of gametangium of *C. psorophorae* in *Cyclops vernalis*. Wall-less gametangium is appressed to host tissue (bottom). Cone shaped nucleus (N), nuclear cap (NC), side body (SB), and basal mitochondrion (M) characteristic of the zoospore of *Coelomomyces* are evident, but obvious cleavage furrows are not evident. ×14,400.

search for the fungus in these copepods revealed the presence of an unknown phase in the life history of *Coelomomyces*. Some of the animals, which had recently ceased swimming, were packed with flagellated swarm cells that will be referred to as planonts (Fig. 1). Dissection of the *Cyclops* indicated that these planonts had been cleaved out of wall-less thalli which had developed in the haemocoel of the host animal (Fig. 2). The active planonts filled all available spaces in the cephalothorax, antennules, legs, and abdomen, and after a variable period of swarming, they escaped to the external environment through a tear in the host's integument. The animal, typically an adult or near adult (4–5th copepodite) died following this traumatic event.

A test of the infectivity of these planonts was made by pooling the planonts from several copepods and distributing them to pans holding the mosquito host (Table 1,C). After 5 days many of the larvae contained hyphal bodies which subsequently developed into the resistant sporangia typical of *C. psorophorae*. These results confirmed that the fungus had two alternate hosts, with the spores from the resistant sporangia infecting the copepods, and the planonts from the copepods infecting the mosquito. Further evidence for this cycle was obtained by exposing the two hosts to the two types of swarmers (Table 1,D). The results indicate that the alternation of different hosts is obligatory; transmission from copepod to copepod or mosquito to mosquito was not seen.

The planonts from the copepod had either one or two posteriorly inserted flagella and their general morphology resembled

TABLE 1. *Infection trials with Coelomomyces psorophorae*

Trial	Potential host	Other components	Infection	Percent infection	Total no. host assayed	No. tests with infection / No. tests attempted
A	Mosquito*	+ RS/Zoospore, + *Cyclops*	Yes	22	212	3/3
	Mosquito	+ RS/Zoospore	No	0	180	0/3
B	Mosquito	+ *Cyclops* (I)†	Yes	54	92	2/2
	Mosquito	+ *Cyclops* (NI)‡	No	0	123	0/2
C	Mosquito	+ Spores from *Cyclops*	Yes	34	137	3/3
	Mosquito		No	0	50	0/1
D	*Cyclops*	+ RS/Zoospore	Yes	32	141§	3/3
	Mosquito	+ RS/Zoospore	No	0	327	0/6
	Mosquito	+ Spores from *Cyclops*	Yes	45	292	6/6
	Cyclops	+ Spores from *Cyclops*	No	0	360§	0/3
	Cyclops	(Control—no spores)	No	0	208§	0/3
	Mosquito	(Control—no spores)	No	0	300	0/6
E	Mosquito	Gametes	No	0	70	0/6¶
	Mosquito	Zygotes and gametes	Yes	30	65	6/6¶

* Mosquito, larvae of *Culiseta inornata*.

† I, *Cyclops* from pans showing infection.

‡ NI, *Cyclops* from pans without record of infection.

§ Adult *Cyclops vernalis*.

¶ Each test used gametes or gamete/zygote populations from different, individual *Cyclops* hosts. Tests run in 100 mm × 50 mm crystalizing dishes.

that of the zoospores from the RS of *C. psorophorae* (Fig. 1). The planonts with a single flagellum were uninucleate and measured 5 × 9 μm, whereas, the biflagellate planonts measured 6 × 12 μm and contained either one or two nuclei. Although improper cleavage could explain this nuclear difference, sexual reproduction, involving uniflagellate isogametes that fuse to form a biflagellate zygote, appeared more likely. This was confirmed by the following observations: (*1*) Dissection of infected copepods at the earliest sign of planont activity provided only uniflagellate planonts. (*2*) Biflagellate planonts appeared after a period of swarming either in or outside the host. (*3*) Uniflagellate planonts (gametes) were seen to fuse and form a biflagellate zygote.

Although most infected copepods released both gametes and zygotes (40–90% zygotes), occasional individuals liberated only gametes which did not undergo fusion. This lack of sexual competence might be explained on the basis of inhospitable mating conditions or the presence of a mating compatibility system. The latter situation appears to be the case. When the persistently uniflagellate populations were combined, zygotes promptly formed in approximately one-half of the crosses. Reciprocal crosses between five different uniflagellate populations suggest that *C. psorophorae* is heterothallic, since all gamete populations that were mutually incompatible fused with each population of the opposite mating type.

The presence of these pure gamete populations allowed us to test the infectivity of the gamete and zygote against mosquito larvae. Larvae in small containers were exposed to gametes or to populations of planonts containing a preponderance of zygotes. The results (Table 1,E) indicate that the gametes will not infect the larvae, but the zygotes, by inference, can.

Microscopical observations of populations of fusing gametes indicate that fusion commences soon after swarming is initiated. Plasmogamy, which occurs at a relatively oblique angle, is quickly followed by reorientation of the basal bodies and flagella until they are closely parallel. The two flagella of active zygotes are so tightly appressed that they appear to be a single unit. Populations examined during the early stages of fusion contain numerous zygotes with two sets of organelles (e.g., nuclei, nuclear caps and side bodies) arranged in a parallel or adjacent manner (Fig. 1). The side bodies coalesce first, followed by fusion of the nuclear caps and nuclei. It seems probable, therefore, that the mosquito larvae are infected by a zygote with a diploid nucleus.

DISCUSSION

The existence of alternate hosts in the life cycle of a parasite is a theme that recurs in many of the major groups of parasitic organisms. In the mycological world, the rust fungi appeared to have a monopoly on this approach to parasitological success. It now appears that the Blastocladiales have evolved their own variation on this theme of heteroecism. The results presented in Table 1 indicate that alternation between mosquito and copepod is obligatory. This conclusion is also supported by the numerous unsuccessful attempts in various laboratories to infect mosquitoes with the zoospores from resistant sporangia. Less study has been devoted to the infection of the copepods, but the infection rate of our copepod-containing "gross" pans tends to diminish quickly if sporangia are not added periodically. This would not be the case if either the gametes or the zygotes were re-infecting the *Cyclops* host.

The water molds included in the Blastocladiales possess a

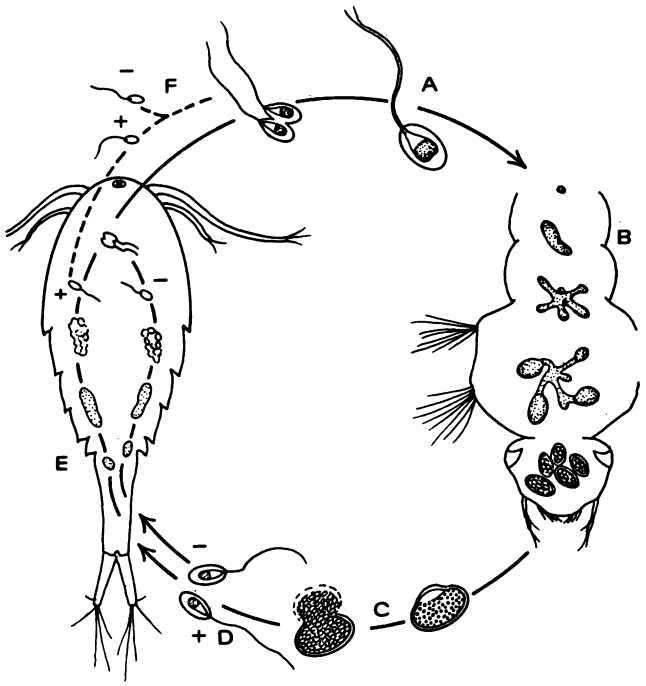

FIG. 3. Life cycle of *C. psorophorae*. Zygote (A) infects larva of *Culiseta inornata* (B) leading to development of hyphal bodies, mycelium and, ultimately, thick-walled resistant sporangia. Under appropriate conditions these sporangia (C) release zoospores of opposite mating type (D) which infect the alternate host, *Cyclops vernalis* (E). Each zoospore develops into a thallus and, eventually, gametangia. Gametes of opposite mating type (F) fuse either in or outside of the copepod to form the mosquito-infecting zygote.

number of unique features that distinguish them from other groups of fungi. These characters include a highly organized zoospore with a membrane-bounded nuclear cap, a resistant sporangium with a multilayered wall, and if sex is involved, fusion between two motile gametes (18). *Coelomomyces* conforms to these characteristics. Emerson (19) has noted three different life cycles in the blastocladialean genus *Allomyces*. The Euallomyces type, with independent sporophyte and gametophyte generations; the Cystogenes type with the gametophyte reduced to a cyst; and the Brachyallomyces type which lacks the gametophyte entirely. The absence of sexuality in the latter type is apparently related to the failure of meiosis in the resistant sporangia. Meiosis occurs in the RS of the first two life history types (20). Subsequent studies on other members of the Blastocladiales [e.g., *Blastocladiella*, *Catenaria*, *Blastocladia* (19, 21–24)] suggest that this pattern of three life histories is widespread in the order and may be recognized as an ordinal characteristic (18).

The development of *C. psorophorae* seems to fit the Euallomyces type of life cycle and displays a number of similarities to the life history of *Blastocladiella variabilis* (21). Although proof that meiosis occurs in the RS is needed, the presence of two vegetative phases separated by isogamy and RS-zoospores provides strong support for the following interpretation of the life history of *C. psorophorae* (Fig. 3). The zygote infects the host mosquito and gives rise to a diploid, coenocytic thallus. After 4 days' growth in the body of the larva, the wall-less mycelium differentiates into thick-walled RS. Thin-walled sporangia, which are found on the sporophyte of *Allomyces* and other members of the Blastocladiales have not been seen in our *C. psorophorae*. They have been reported, however, in

mosquitoes infected with other species of *Coelomomyces* (8). If the mature resistant sporangia are placed in appropriate conditions, they will germinate by the two-step germination process characteristic of the genus. Based on comparisons to other genera of the order, we assume that meiosis occurs during germination of the RS. Preliminary aceto-orcein squashes indicate that nuclear divisions are occurring at this time but the problems presented by the small size of the chromosomes call for further work with a variety of cytological techniques. After infecting the copepod, the RS-zoospore, or meiospore, develops into a haploid thallus which eventually differentiates gametes of the appropriate mating type. The cell wall is absent during gametogenesis and the isogametes are in an advanced state of differentiation before cleavage is initiated (Fig. 2). If the host contains thalli of opposite mating type, their gametes may fuse during the swarming period inside the host animal; if not, they must seek their mate outside the copepod. Further development of the cycle depends on the zygote finding and infecting a new mosquito host. Infection studies in the laboratory have followed the same fungal material from an infected mosquito, through the intermediate copepod host and back to a new mosquito. This observation precludes the existence of other unknown hosts in this life history.

The minimum time to complete the cycle in our rearing conditions is approximately 20 days, with 9 days from spore to RS in the mosquito, 4 days for maturation of the RS, and 7 days from meiospore to gametes in the copepods.

The copepod phase of *C. psorophorae* is remarkably similar to the *Cyclops* parasite, *Callimastix cyclopis*. Originally described as a protozoan parasite of *Cyclops* (25), this organism has more recently been associated with the Blastocladiales (26, 27). The few differences that exist between the two genera may well relate to different host species and rearing conditions. Problems of synonomy and taxonomic priority between *Coelomomyces* (1921) and *Callimastix* (1912) must await resolution of these differences and study of any available type material.

Whether the other forty varieties (1, 28) and species of *Coelomomyces* follow the full cycle described here for *C. psorophorae* awaits study. Discovery of "brachy" and "cystogenes" cycles would not be surprising and these would presumably lack the crustacean host. However, the problems of laboratory domestication encountered by workers with other host-parasite combinations could argue for the existence of long-cycle forms in other species of the fungus.

The discovery of heteroecism in *Coelomomyces* has obvious implications for the potential use of this fungus in biological control of mosquitoes and other dipteran hosts. Knowledge of the copepod involvement has permitted us, after a long period of erratic results, to obtain consistently high levels of mosquito mortality with relatively few infected copepods. The discovery of sex and mating type presents a potential tool for examining the genetics of infectivity and speciation in the genus as well as selection for "super killers."

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1. Couch, J. N. & Umphlett, C. J. (1963) in *Insect Pathology: An Advanced Treatise*, ed. Steinhaus, E. A. (Academic Press, New York), Vol. 2, pp. 149–188.
2. Garnham, P. C. C. & Lewis, D. J. (1959) "Parasites of British Honduras with special reference to leishmaniasis," *Trans. Roy. Soc. Trop. Med. Hyg.* **53**, 12–35.
3. Weiser, J. & Vávra, J. (1964) "Zur Verbreitung der *Coelomomyces*-Pilze in europäischen Insekten," *Z. Tropenmed. Parasitol.* **15**, 38–42.
4. Koval, E. Z. & Andreeva, R. V. (1971) "On studying pathogenic micoflora of horse flies," *Dopov. Akad. Nauk. Ukr. RSR Ser. B* **11**, 1042–1044.
5. Manier, J.-F., Rioux, J.-A., Coste, F. & Maurand, J. (1970) "*Coelomomyces tuzetae* n. sp. (Blastocladiales-Coelomomycetaceae) parasite des larves de Chironomes (Diptera-Chironomidae)," *Ann. Parasitol.* **45**, 119–128.
6. Whisler, H. C., Shemanchuk, J. A. & Travland, L. B. (1972) "Germination of the resistant sporangia of *Coelomomyces psorophorae*," *J. Invert. Pathol.* **19**, 139–147.
7. Walker, A. J. (1938) "Fungal infections of mosquitoes, especially of *Anopheles costalis*," *Ann. Trop. Med. Parasitol.* **32**, 231–244.
8. Muspratt, J. (1946) "On *Coelomomyces* fungi causing high mortality of *Anopheles gambiae* larvae in Rhodesia," *Ann. Trop. Med. Parasitol.* **40**, 10–17.
9. Muspratt, J. (1946) "Experimental infection of the larvae of *Anopheles gambiae* (Dipt., Culicidae) with a *Coelomomyces* fungus," *Nature* **158**, 202.
10. Shemanchuk, J. A. (1959) "Note on *Coelomomyces psorophorae* Couch, a fungus parasitic on mosquito larvae," *Can. Entomol.* **91**, 743–744.
11. Laird, M. (1967) "A coral island experiment: a new approach to mosquito control," *World Health Organ. Chron.* **21**, 18–26.
12. Couch, J. N. (1972) "Mass production of *Coelomomyces*, a fungus that kills mosquitoes," *Proc. Nat. Acad. Sci. USA* **69**, 2043–2047.
13. Whisler, H. C., Zebold, S. L. & Shemanchuk, J. A. (1974) "Alternate host for mosquito parasite *Coelomomyces*," *Nature* **251**, 715–716.
14. McLintock, J. (1952) "Continuous laboratory rearing of *Culiseta inornata* (Will.) (Diptera: Culicidae)," *Mosquito News* **12**, 195–201.
15. Whisler, H. C. (1966) "Host-integrated development in the Amoebidiales," *J. Protozool.* **13**, 183–188.
16. Umphlett, C. J. & Olson, L. W. (1967) "Cytological and morphological studies of a new species of *Phlyctochytrium*," *Mycologia* **59**, 1085–1096.
17. Whisler, H. C. & Fuller, M. S. (1968) "Preliminary observations on the holdfast of *Amoebidium parsiticum*," *Mycologia* **60**, 1068–1079.
18. Sparrow, F. K. (1960) *Aquatic Phycomycetes* (University of Michigan Press, Ann Arbor), 2nd ed.
19. Emerson, R. (1941) "An experimental study of the life cycles and taxonomy of *Allomyces*," *Lloydia* **4**, 77–144.
20. Wilson, C. M. (1952) "Meiosis in Allomyces," *Bull. Torrey Bot. Club* **79**, 139–160.
21. Harder, R. & Sörgel, G. (1938) "Über einen neuen planoisogamen Phycomyceten mit Generationswechsel und seine phylogenetische Bedeutung," *Nachr. Ges. Wiss. Göttingen* **3**, 119–127.
22. Couch, J. N. & Whiffen, A. J. (1942) "Observations on the genus *Blastocladiella*," *Amer. J. Bot.* **29**, 582–591.
23. Couch, J. N. (1945) "Observations on the genus *Catenaria*," *Mycologia* **37**, 163–193.
24. Blackwell, E. M. (1940) "A life cycle of *Blastocladia pringsheimii* Reinsch.," *Trans. Brit. Mycol. Soc.* **24**, 68–86.
25. Weissenberg, R. (1912) "*Callimastix cyclopis*, n. g., n. sp., ein geisselträgendes Protozoon aus dem Serum von *Cyclops*." *Sitzungsber. Ges. Naturfr. Freunde* **5**, 299–305.
26. Weissenberg, R. (1950) "The development and affinities of *Callimastix cyclopis* Weissenberg, a parasitic microorganism from the serum of *Cyclops*," *Proc. Amer. Soc. Protozool.* **1**, 4–5.
27. Vávra, J. & Joyon, L. (1966) "Étude sur la morphologie, le cycle évolutif et la position systématique de *Callimastix cyclopis* Weissenberg 1912," *Protistologia* **2**, 5–16.
28. Roberts, D. W. (1974) in *Mosquito Control*, ed. Bourassa, J.-P. (Univ. of Quebec Press, Montreal), in press.