PHEROMONE RACES OF *OSTRINIA NUBILALIS* HÜBNER (LEPIDOPTERA: CRAMBIDAE) INFESTING GRAIN CORN IN MANITOBA, ONTARIO, AND QUÉBEC PROVINCES OF CANADA

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Abstract

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Ostrinia nubilalis (Hübner) (Lepidoptera: Crambidae), European corn borer, is an economic pest of *Zea mays* (Linnaeus) (Poaceae) and other vegetable crops that is distributed throughout the agricultural production regions of Ontario, Québec, and Manitoba in Canada. Two phenotypic races of *O. nubilalis* have been identified that differ in the proportion of isomers of 11-tetradecenyl acetate (11-14:OAc) in their sex pheromone. The Z-race (Z-11-14:OAc) is the predominant race in the United States of America, known to inhabit *Zea mays* as its primary host, whereas the E-race (E-11-14:OAc) infests a wider host range, including many vegetable crops, and is only found within the Eastern coastal states of the United States of America. Collections of *O. nubilalis* were made from grain corn in agricultural regions of Ontario, Québec, and Manitoba in 1997, 2008, 2009, and 2010, and females were analyzed for pheromone race using gas chromatography (GC). Only Z-race *O. nubilalis* were found in Ontario (from Essex to Leeds and Grenville Counties) and in Southern Manitoba. E-race individuals were detected in collections from Ottawa, Ontario and St. Anicet, Québec, with an increasing proportion of E-race phenotypes in samples from west to east. This is the first report of pheromone race determination using GC among Canadian *O. nubilalis* populations and the first documentation of E-race *O. nubilalis* in Canada using GC.

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Introduction

Ostrinia nubilalis (Hübner) (Lepidoptera: Crambidae), European corn borer, has been an economic pest of corn, *Zea mays* (Linnaeus) Poaceae, throughout North America since introduction early in the 20th century (Caffrey and Worthley 1927; Mason *et al*. 1996). Two phenotypic races of this species have been identified that differ in the proportion of 11-tetradecenyl acetate (11-14:OAc) geometrical isomers in their sex pheromone composition (Klun and Brindley 1970). Although *O. nubilalis* is reported to utilize over 200 host plants, it predominantly infests corn, as its common name implies; however, the E-race (E-11-14:OAc) inhabits a wider host range, including peppers *Capsicum* spp. (Linnaeus) Solanaceae, potato *Solanum tuberosum* (Linnaeus) Solanaceae, tomato *Solanum lycopersicum* (Linnaeus) Solanaceae, and wheat *Triticum aestivum* (Linnaeus) Poaceae, as well as corn, whereas the Z-race (Z-11-14:OAc) has a strong fidelity to corn (Bontemps *et al*. 2004; Mason *et al*. 1996). The Z-race is present throughout the North American range of *O. nubilalis* (Palmer *et al*. 1985); populations within the United States of America Corn belt are dominated by the Z-race (Mason *et al.* 1996; Showers *et al.* 1974), whereas the Northeastern coastal states contain greater proportions of the E-race (Klun and Brindley 1970; Mason *et al.* 1996; O'Rourke *et al.* 2010; Roelofs *et al.* 1972; Roelofs *et al.* 1985). Regional pheromone race identification of *O. nubilalis* is important for effective integrated pest management in agricultural crops including population monitoring using pheromone traps (DuRant *et al.* 1995) and for resistance management implications (Bontemps *et al.* 2004; O'Rourke *et al.* 2010). The major corn producing areas in Canada are in southern portions of Manitoba, Ontario, and Québec (Hamel and Dorff 2013); however, the pheromone race composition of *O. nubilalis* from these regions has not been reported.

Klun *et al.* (1975) reported results for captures of *O. nubilalis* males in pheromone traps from several locations in Canada. They tested blends of the two pheromone isomers ranging from dominance of Z at one extreme to dominance of E in the lures at the other end of the spectrum. Their trapping data showed that more than 15% of males trapped at Simcoe, Ontario and St. Jean, Québec were attracted to E pheromone blends out of a total catch of at least 45 moths in each case. At three other locations, moths predominantly were caught in traps baited with Z blends. Although pheromone trapping of males can provide an indication of presence of E and Z races in a population, this method is not definitive because males exhibit different levels of response to E and Z independently baited lures (Glover *et al*. 1991; Mason *et al*. 1997; Pelozuelo and Frérot 2007). McLeod *et al*. (1979) reported that male *O. nubilalis* collected from two Ontario locations and one population from St. Rémi, Québec responded most strongly to Z-11-14:OAc using electroantennograms. However, another population that infested corn later in the same growing season from the Québec location responded with greater affinity to E-11-14:OAc. The most reliable method of race determination is analysis through gas chromatography of excised female pheromone glands or by analysis of race-specific single nucleotide polymorphism (SNP) genetic markers (Coates *et al*. 2013).

Although grain corn is the second largest crop produced in Ontario by acreage, vegetable crops such as field tomatoes, sweet corn, and peppers also provide substantial farm value to the agricultural economy within the province (Hagerman 1997). The presence of significant acreages of fruit and vegetable crops in Essex, Chatham-Kent, and Niagara

Counties in Ontario, which have the potential to support E-race *O. nubilalis*, and reports of infestation of winter wheat *T. aestivum* (Linnaeus) Poaceae in Québec and eastern Ontario (F. Meloche, personal communication) prompted the investigation of the composition of pheromone races in Canadian populations of *O. nubilalis*. Although Klun *et al.* (1975) and McLeod (1979) provided results for males collected in E- and Z-baited traps, the pheromone composition has not been documented with race-specific analysis through gas chromatography or SNP analysis. Although there is some hybridization in the field, E and Z populations are usually isolated due to multiple reproductive barriers (Dopman *et al*. 2010). The present study represents the first report of race-specific testing of Canadian *O. nubilalis* populations using gas chromatography; these results were generated prior to the development and publication of methods for SNP analysis (Coates *et al*. 2013). Populations of *O. nubilalis* were collected from commercial grain corn fields in Ontario, Québec, and Manitoba in 1997, 2008, 2009, and 2010, and sent to C.E.M. at the University of Delaware for pheromone gland analysis of females using gas chromatography.

Materials and Methods

Insect Specimens

O. nubilalis larvae were collected in September or October of each sampling year from commercial grain corn fields that had not been planted with transgenic hybrids that express *Bacillus thuringiensis* (Berliner) (*Bt*) Bacillales insecticidal proteins (*Bt*-corn) or from non-*Bt* refuge plants within *Bt*-corn fields (Table 1, Fig. 1). In 1997, 50 field-collected larvae from each location were cooled and directly shipped, in cardboard larval rearing rings with artificial diet, to C.E.M. for pheromone analysis. In 2008, 2009, and 2010, corn stalks containing diapausing larvae were removed from growers' fields and kept over winter in a non-heated barn at the University of Guelph Ridgetown Campus (Ridgetown, Ontario). Following termination of diapause, larvae were extracted from the corn stalks and transferred into rearing dishes with cardboard pupation rings, which were placed in growth chambers maintained at 16:8 L:D, 27 °C photoperiod, 18 °C scotoperiod, and 75 % relative humidity (RH) to establish laboratory colonies; original colony sizes ranged from 20–70 individuals. After multiple generations of laboratory rearing (Table 1), pupae were removed from the colony, sexed, and female pupae were shipped to C.E.M. for pheromone analysis.

Upon receipt by C.E.M., individual larvae and/or pupae were housed in 28 ml plastic food service cups containing cotton rolls saturated with water, and these were placed in a growth chamber set on a reversed photoperiod to facilitate gland removal at regular working hours. Through pupation and eclosion, conditions were set at 25 °C, 16:8 (L:D) photoperiod, and 50–80 % RH. Drinking water was provided for newly emerged moths, and females were set aside for pheromone analysis.

Pheromone ring glands of females were excised with micro-scissors at the non-sclerotized terminal segment, just anterior of the single ring gland, during the 7th h of scotophase the second day after eclosion (24–48 h old). Each gland was placed into a 50-µl point-tipped auto-sampler vial containing 5 µl of heptane and an internal standard of 4.5 ng cis-7-tetradecenyl acetate (Z-7-14:OAc). Samples were held for ≥ 30 min at room temperature or stored in a − 20 °C freezer before analysis.

TABLE 1. Location of Canadian field collections of *Ostrinia nubilalis* in 1997, 2008, 2009, and 2010, and the percentage of E and Z pheromone races or hybrids determined using gas chromatography (GC).

County/Regional Municipality	Nearest town	Geographic coordinates		Year of collection	Generation used in GC analysis	n[1]	Percentage		
		Latitude	Longitude				E	Z	Hybrid
Ontario									
Essex	Harrow	42.0021	-82.8182	2010	F12,14	28	0.0	100.0	0.0
Chatham-Kent	Ridgetown	42.2711	-81.5319	1997	F0	37	0.0	100.0	0.0
Chatham-Kent	Ridgetown	42.2711	-81.5319	2010	F14	13	0.0	100.0	0.0
Middlesex	London	42.9757	-81.1052	1997	F0	21	0.0	100.0	0.0
Huron	Brussels	43.7428	-81.2429	1997	F0	31	0.0	100.0	0.0
Niagara	Winger	42.9430	-79.4298	2008	F26	11	0.0	100.0	0.0
Niagara	Winger	42.9474	-79.3879	2010	F9	16	0.0	100.0	0.0
Leeds and Grenville	Kemptville	44.8478	-75.5509	1997	F0	21	0.0	100.0	0.0
Ottawa-Carleton	Ottawa	45.2313	-75.4337	2008	F25	14	7.1	71.4	21.4
Manitoba									
Grey	Elm Creek	49.7317	-98.0623	2009	F17	15	0.0	100.0	0.0
Québec									
Le Haut-Saint-Laurent	St. Anicet	45.1062	-74.3361	2008	F25	15	20.0	13.3	66.7

[1] Number of females tested with sufficient quantity of pheromone to produce GC peaks at the appropriate retention time. Peak height consisting of ≥ 95% E isomer compared to the Z isomer were classified as E-race, those with ≤ 5% E isomer were classified as Z-race, and those with intermediate percentages of E isomer (20-80%) were classified as hybrids.

Gas Chromatography

Pheromone extractions were analyzed with a Varian 3500 gas chromatograph equipped with a Varian 8200 auto-sampler (Agilent Technologies, Santa Clara, California, United States of America) using capillary techniques similar to those described by Field *et al.* (1999) and DuRant *et al.* (1995). Three µl of solution from the sample were injected in the gas chromatograph injector using a sandwich technique where a 0.5 µl upper air gap was placed between the solvent plug and sample plug in a 10-µl syringe. The air gap resides between the sample and the solvent that remains below the syringe plunger after rinsing the syringe. The air gap prevents liquid-to-liquid contact and reduces the chance of sample contamination from previous samples. A 0.8 µl lower air gap was used to reduce sample volatilization during insertion of the needle into the hot injector.

During injection, the hot needle time was zero, the injection rate was 1.5 µl sec^{-1}, and the needle residence time was 0.02 min. The gas chromatograph was equipped with a heated injector fitted with a 4 mm inside diameter open-top glass uniliner (Restek Corporation, Bellefonte, Pennsylvania, United States of America) containing glass wool, a fused silica capillary column (15 m x 0.25 mm) with 0.25 µm Stabilwax® film thickness (Restek Corporation, Bellefonte, Pennsylvania, United States of America), a 5 m x 0.25 mm fused silica guard column, and a flame ionization detector. The gas chromatograph was programmed as follows: injector temp: 200 °C, splitless for 1.5 min, then set to split for the remainder of the run (split ratio 50:1 set at 60 °C); detector temp: 250 °C, attenuation set at 32 $\times 10^{-11}$; column oven programmed at 80 °C, held 2.0 min, heat from 80 °C to 240 °C at 10 °C min^{-1}, held at 240 °C for 5 min to end of the run; and total run time was 23 min. Hydrogen was used as carrier gas at a flow rate of 20 cm sec^{-1} (6.5 psi head pressure) and nitrogen was used as makeup gas. Under these conditions, the Z-7-14:OAc internal standard and the two pheromone isomers eluted at ≈ 13.1 – 13.5 min with each of the three peaks being separated by 0.2 to 0.4 min, which allowed for distinct separation on the chromatogram and detection of these compounds (Fig. 2).

FIGURE 1. Locations where *Ostrinia nubilalis* tested for pheromone race analysis were collected in 1997, 2008, 2009, and 2010 in Canada.

Chromatogram results for female moths for which pheromone glands were excised and analyzed by gas chromatography were categorized by pheromone race based on the percentage ratio of the two pheromone isomers. The percentages were determined by comparison of peak heights of the isomers at the appropriate retention times on the chromatogram. Samples with the peak height consisting of 95 % or more of the E isomer compared to the Z isomer were classified as E-race, those with 5 % or less of the E isomer were classified as Z-race, and those with intermediate percentages of E isomer were classified as hybrids, whereby the percentages with very few exceptions fell within the 20 % to 80 % range. Although these criteria have a broad range, analyses by C.E.M. of approximately 1000 *O. nubilalis* showed that E and Z phenotypes do not fall outside the 5 % range for the minor isomer of each phenotype (Coates *et al*. 2013). Allelic variation in a fatty-acyl reductase gene essential for pheromone biosynthesis accounts for the phenotypic variation in female pheromone production (Lassance *et al*. 2010). Mean percentages and standard deviations for the E isomer are typically about 98.5 ± 0.5 % for the E race, 67 ± 10 % for the hybrid, and 3 ± 1.0 % for the Z race.

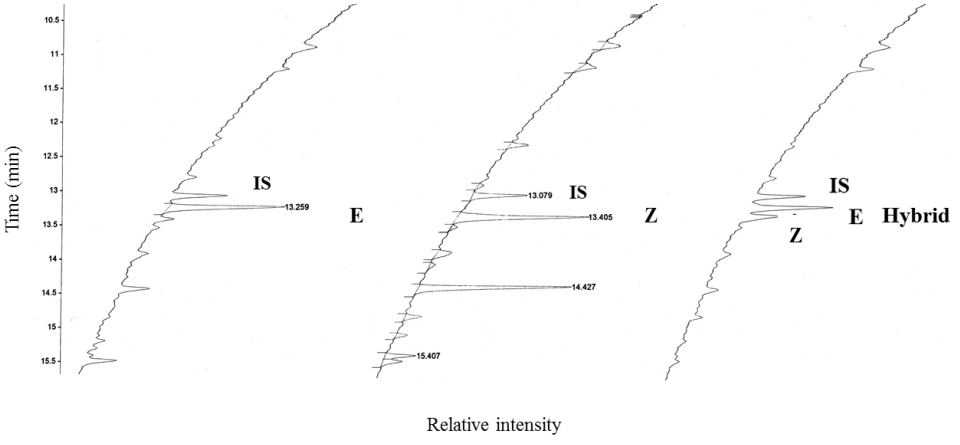

FIGURE 2. Representative chromatograms from pheromone analyses of *Ostrinia nubilalis* in Canada showing peaks for internal standard (IS), Z-11-14:Oac (Z), and E-11-14:Oac (E).

Results and Discussion

In all years of sampling, only Z-race *O. nubilalis* were found in collections from grain corn in Essex, Chatham-Kent, Middlesex, Huron, Niagara, Leeds and Grenville Counties in Ontario, and from Grey County in Manitoba (Table 1, Fig. 1). Both E- and Z-race phenotypes were identified in samples collected in grain corn from Ottawa, Ontario and Saint-Anicet, Québec (Table 1). The Ottawa population collected in 2008 contained 21.4 % hybrid females where significant quantities of both E and Z isomers were measured by gas chromatography; however, the majority of individuals were Z-race (71.4 %) and 7.1 % were E-race (Table 1). Of the individuals tested from Québec, the majority tested were E

and Z hybrids (66.7 %), and there was a greater proportion of E-race insects (20.0 %) than Z-race (13.3 %) (Table 1).

Our results show that the Z-race of *O. nubilalis* is the dominant pheromone race found infesting grain corn in the major corn producing regions of Canada, present throughout Ontario from Essex to Ottawa Counties and in southern Manitoba. E-race *O. nubilalis* were only present in colonies derived from collections from grain corn in eastern Ontario near Ottawa and Québec, which indicates the presence of E-race within these regions. The proportion of Z or E races among founder males and females from original field collections and the resulting frequency of hybridization among offspring prior to GC analysis are unknown. Detection of E-11-14:OAc isomers in our analysis is evidence that E-race *O. nubilalis* were originally present in the area sampled. However, where no E-11-14:OAc isomers were detected there is a degree of uncertainty as to whether the E-race was lost through generations of rearing in the laboratory or because the small number of founder individuals in some collections may not have been sufficient to detect E-race individuals. A relatively new method of distinguishing pheromone races of *O. nubilalis* using SNP markers enables high throughput processing of larger sample sizes and has greater than 98 % correlation with results from GC analysis (Coates *et al*. 2013; Lassance *et al*. 2010). Therefore we are confident in our results indicating E- and Z-race phenotypes.

Klun *et al.* (1975) reported some males collected in E-race pheromone traps at Chatham and Simcoe, Ontario and St. Jean, Québec. It is possible that some E-race *O. nubilalis* were present in these areas in 1973 and 1974 when Klun *et al.* (1975) conducted their study. Our results indicate it is unlikely that the E-race is currently present in southwestern Ontario. Since we found the E-race phenotype present in the eastern range of populations we studied, it is likely that the E-race is present further east from St. Anicet, Québec and Ottawa, Ontario. Although we did not show the presence of the E-race phenotype at the Kemptville collection site, the E-race may be present there now since the samples we analyzed were from a decade earlier in the 1997 collection; however, testing of current populations must be completed for confirmation. A more in-depth study of populations of *O. nubilalis* collected from a wider host range within the regions studied would provide more conclusive information on the pheromone race composition of *O. nubilalis* in Canada. This is the first documented evidence of the E-race in Canada corresponding with the eastern corn growing region, which is a similar distribution pattern as in the U.S. These results provide useful information for pheromone trap monitoring of *O. nubilalis* in Eastern Ontario and Québec, and support observations of infestations in non-corn crops. *O. nubilalis* is also a significant pest of potato in Québec, New Brunswick, and Prince Edward Island (Noronha *et al.* 2008). Consequently, the E-race of *O. nubilalis* very well could be prominent in these areas east of where we conducted our study. Further analysis of populations collected from these regions is needed to determine this.

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References

Bontemps, A., Bourguet, D., Pélozuelo, L., Bethenod, M.-T., and Ponsard, S. 2004. Managing the Evolution of *Bacillus thuringiensis* Resistance in Natural Populations of the European Corn Borer, *Ostrinia nubilalis*: Host Plant, Host Race and Pherotype of Adult Males at Aggregation Sites. *Proceedings: Biological Sciences* **271**: 2179–2185. doi:10.1098/rspb.2004.2851

Caffrey, D. J. and Worthley, L. H. 1927. A progress report on the investigations of the European corn borer. *Edited by* U. S. D. o. Agriculture, Washington, D.C., U.S.A. Pp. 1–162.

Coates, B. S., Johnson, H., Kim, K.-S., Hellmich, R. L., Abel, C. A., Mason, C., and Sappington, T. W. 2013. Frequency of hybridization between *Ostrinia nubilalis* E- and Z-pheromone races in regions of sympatry within the United States. *Ecology and Evolution* **3**: 2459–2470. doi:10.1002/ece3.639

Dopman, E. B., Robbins, P. S., and Abby, S. 2010. Components of reproductive isolation between North American pheromone strains of the European corn borer. *Evolution* **64**: 881–902. doi:10.1111/j.1558-5646.2009.00883.x

DuRant, J. A., Fescemyer, H. W., Mason, C. E., and Udayagiri, S. 1995. Effectiveness of four blends of European corn borer sex pheromone isomers at three locations in South Carolina. *Journal of Agricultural Entomology* **12**: 241–253.

Field, L. M., James, A. A., Marçon, P. C. R. G., Taylor, D. B., Mason, C. E., Hellmich, R. L., and Siegfried, B. D. 1999. Genetic similarity among pheromone and voltinism races of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). *Insect Molecular Biology* **8**: 213–221. doi:10.1046/j.1365-2583.1999.820213.x

Glover, T. J., Knodel, J. J., Robbins, P. S., Eckenrode, C. J., and Roelofs, W. L. 1991. Gene Flow Among Three Races of European Corn Borers (Lepidoptera: Pyralidae) in New York State. *Environmental Entomology* **20**: 1356–1362. doi:10.1093/ee/20.5.1356

Hagerman, P. 1997. European corn borer in sweet corn and other horticultural crops. [online] Available from http://www.omafra.gov.on.ca/english/crops/facts/97-019.htm [accessed May 22, 2014].

Hamel, M. and Dorff, E. 2013. Corn: Canada's third most valuable crop. [online] Available from http://www.statcan.gc.ca/pub/96-325-x/2014001/article/11913-eng.htm [accessed June 2, 2014].

Klun, J. A. and Brindley, T. A. 1970. cis-11-Tetradecenyl Acetate, a Sex Stimulant of the European Corn Borer. *Journal of Economic Entomology* **63**: 779–780. doi:10.1093/jee/63.3.779

Klun, J. A. 1975. Insect Sex Pheromones: Intraspecific Pheromonal Variability of *Ostrinia nubilalis* in North America and Europe. *Environmental Entomology* **4**: 891–894. doi:10.1093/ee/4.6.891

Lassance, J.-M., Groot, A. T., Lienard, M. A., Antony, B., Borgwardt, C., Andersson, F., Hedenstrom, E., Heckel, D. G., and Lofstedt, C. 2010. Allelic variation in a fatty-acyl reductase gene causes divergence in moth sex pheromones. *Nature* **466**: 486–489. doi:10.1038/nature09058

Mason, C. E., Eheresman, N. P., He, K., Ilalia, A. D., and Pesek, J. D. 1997. Performance of three commercial pheromone sources for trapping European corn borer. *In* Ingerson-Mahar, J., (ed). *31st Northeast Regional Field Crops Insect Conference at Burlington*, VT, Rutgers University, New Brunswick, NJ. Pp. 27–34.

Mason, C. E., Rice, M. E., Calvin, D. D., Van Duyn, J. W., Showers, W. B., Hutchison, W. D., Witkowski, J. F., Higgins, R. A., Onstad, D. W., and Dively, G. P. 1996. European Corn Borer Ecology and Management. North Central Regional Extension Publication, Ames, Iowa.

McLeod, D. G. R., Ritchot, C., and Nagai, T. 1979. Occurrence of a two generation strain of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), in Quebec. *The Canadian Entomologist* **111**: 233–236. doi:10.4039/Ent111233-3

Noronha, C., Vernon, R. S.,and Vincent, C. 2008. Key pests of potatoes in Canada. *Cahiers Agricultures* **17**: 375–381.

O'Rourke, M. E., Sappington, T. W., and Fleischer, S. J. 2010. Managing resistance to Bt crops in a genetically variable insect herbivore, *Ostrinia nubilalis*. *Ecological Applications* **20**: 1228–1236. doi:10.1890/09-0067.1

Palmer, D. F., Schenk, T. C., and Chiang, H. C. 1985. Dispersal and Voltinism Adaptation of the European Corn Borer in North America, 1917-1977. University of Minnesota, Minneapolis, MN, USA. Pp. 31.

Laurent, P. and Frérot, B. 2007. Monitoring of European Corn Borer with Pheromone-Baited Traps: Review of Trapping System Basics and Remaining Problems. *Journal of Economic Entomology* **100**: 1797–1807. doi:10.1603/0022-0493(2007)100[1797:MOECBW]2.0.CO;2

Roelofs, W. L., Carde, R. T., Bartell, R. J., and Tierney, P. G. 1972. Sex Attractant Trapping of the European Corn Borer in New York. *Environmental Entomology* **1**: 606–608. doi:10.1093/ee/1.5.606

Roelofs, W. L., Du, J. W., Tang, X. H., Robbins, P. S., and Eckenrode, C. J. 1985. Three European corn borer populations in New York based on sex pheromones and voltinism. *Journal of Chemical Ecology* **11**: 829–836. doi:10.1007/bf01012071

Showers, W. B., Reed, G. L., and Oloumi-Sadeghi, H. 1974. European Corn Borer: Attraction of Males to Synthetic Lure and to Females of Different Strains. *Environmental Entomology* **3**: 51–58. doi:10.1093/ee/3.1.51