and the fluorescence increased. In contrast, DCB-treated protoplasts remained spherical and no fluorescence could be noted. But except for their form, they apparently behaved like non-treated protoplasts and after 5–7 days of culture, divisions occurred (fig., A and B).

Similar observations concerning DCB effects on protoplasts have previously been reported, the absence of the wall being proved by electron microscopy[8]. As for ethylene production, no differences were obtained between control and DCB-treated protoplasts, i.e. between protoplasts with or without a new wall. Ethylene formation was quite linear during the first 3 days, then decreased until the 6 th day (table 1). DCB itself did not interfere with ethylene metabolism, as could be concluded from the data reported in table 2, for which tissues were tested instead of protoplasts.

In conclusion, ethylene production by protoplasts seems not to be linked to the synthesis of a new wall, which cannot regulate ethylene production at least for protoplasts.

Table 2. Ethylene production by mesophyll tissues cultivated in media with or without dichlorobenzonitrile (DCB)

Duration of culture (h)	Ethylene production (ppb × 10^3 per g of fresh weigt, ± SD) Without DBC (control)	With DBC
24	3.5±0.4	3.7±0.4
48	9.3±1.1	8.7±1.3
72	17.7±2.9	17.9±3.0

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Trace co-attractants in synthetic sex lures for 22 noctuid moths

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Summary. Trace components contributed significantly to the potency of synthetic sex attractant lures for males of many species of Noctuidae. Improved synthetic blends for 12 moths including *Euxoa ochrogaster* and *Trichoplusia ni*, and new lure blends for 10 moths are described. In every case the trace constituents were structural analogs of the main lure components.

Sex attractants for lepidoptera are recognized as blends rather than single biologically active chemicals, noctuid moth pheromones affording many examples of multi-component systems[1]. Lure blends which involve trace components were demonstrated in the sex pheromones of the tobacco budworm *Heliothis virescens* and the corn earworm *H. zea*[2]. More recently, trace co-attractants have been reported in sex pheromones of the armyworm *Pseudaletia unipuncta*[3,4] and the red-backed cutworm *Euxoa ochrogaster*[5–7]. We now report new or improved synthetic sex attractant blends, containing trace co-attractants, for males of 22 noctuid species.

Materials and methods. High-purity (> 99%) chemicals were used, synthesized by recognized methods. Assays by capillary gas chromatography[7] showed that, to the detection limit of 0.01%, each compound was free of geometrical isomers, positional isomers, homologs and analog impurities. Replicated tests were carried out using double-cone traps[8] which required moths to land prior to trap entry and thus provided some measurement of attraction at close range in the later stages of the male response sequence[9]. Red rubber septa (5×9 mm) were employed as blend dispensers, lure materials being impregnated into the septa via dilute solutions in hexane.

Trace co-attractants were mostly demonstrated by 2-stage field screening experiments. In the 1st stage a synthetic compound or blend known to lure target species to traps was admixed with 10% of each of about 30 analogs including *(Z)*-5-decenyl acetate and the primary alcohols, acetates and aldehydes based on the alkenyl groups *(Z)*-5-dodecenyl, *(Z)*-7-dodecenyl, *(Z)*-9-dodecenyl, *(Z)*-5-tetradecenyl, *(Z)*-7-tetradecenyl, *(Z)*-9-tetradecenyl, *(Z)*-11-tetradecenyl, *(Z)*-7-hexadecenyl, *(Z)*-9-hexadecenyl and *(Z)*-11-hexadecenyl. This array constitutes the main range of biologically active monoolefins known in noctuid sex attractants[10,11]. Those analogs which eliminated or greatly suppressed trapping were re-tested at 1% and 0.1% levels of admixture in the 2nd stage of the screening. The rationale for initial testing at 10% levels was limitation of the number of chemicals for subsequent tests to those which inhibited trapping when present in substantial amounts in the lure. Such compounds, inhibitory at 10%, might be true trapping inhibitors which suppress trapping at all levels of addition to a lure, or might be trace co-attractants producing trapping suppression only at super-optimal levels[12,13]. In this way many candidate trace co-attractants could be identified, inhibitory effects at 10% being easily observed among a large number of non-inhibiting treatments. In subsequent trapping tests where the inhibitors were added in trace amounts (0.1–1%) to the lures, those associated with trapping significantly above controls were considered to be trace co-attractants and were then subjected to further replicated trapping tests to determine the most effective blend ratios. Trace effects in a few species were discovered by chance during examination of other target moths. In every instance ancillary dose/capture experiments were performed to ensure that blend ratios were optimized at near-optimal blend release rates. Species having optimal blend ratios smaller than 100:1 were not considered to qualify as 'trace' systems and are not included in the tables. Standard abbreviations for attractant chemicals are used in the tables and below: thus Z7-12:Ac = *(Z)*-7-dodecenyl acetate, Z9-14:Ald = *(Z)*-9-tetradecenal, Z11-16:OH = *(Z)*-11-hexadecenol, and so forth.

Results and discussion. Trace co-attractant systems were defined and field proven for 22 noctuids representing 5 sub-families, showing that such systems are general and widespread in the Noctuidae. Typically, inclusion of the

Table 1. Sex attractants involving trace components for 22 noctuid moths. Starred compounds are known constituents of the female sex pheromone. Species for which no lure has previously been reported are preceded by (N)

	Species	Attractant blend (optimum ratio)	Optimum dose, µg	Increase in attractancy[a]
(N)	*Abagrotis placida* Grote	Z7-14:Ac + Z11-14:Ac (10000:1)	1000–2000	2
	Agrotis venerabilis Walker	Z5-10:Ac + Z7-12:Ac + Z5-12:Ac (100:10:1)	10–50	3
(N)	*Apamea indela* Smith	Z11-16:Ac + Z11-16:OH + Z11-16:Ald (200:50:1)	ca. 500	> 100
	Chrysaspidia putnami Grote	Z5-12:Ac + Z7-12:Ac (100:1)	50–200	8
(N)	*Enargia infumata* Grote	Z11-16:Ac + Z9-14:Ac (500:1)	500–2000	> 50
	Eurois occulta Linnaeus	Z9-14:Ac + Z11-16:Ac + Z11-16:Ald (100:30:1)	500–2000	3
	Euxoa flavicollis Smith	Z9-14:Ac + Z7-12:Ac (500:1)	500–2000	6
	Euxoa obeliscoides Grote	Z9-14:Ac + Z7-12:Ac (500:1)	50–200	30
	Euxoa ochrogaster Guenée	Z5-12:Ac* + Z7-12:Ac* + Z9-12:Ac* + Z5-10:Ac (400:4:1:1)	100–500	(next table)
(N)	*Homohadena infixa* Walker	Z7-14:Ac + Z9-14:Ac (200:1)	ca. 500	> 100
(N)	*Ipimorpha pleonectusa* Grote	Z11-16:Ald + Z11-16:OH (100:1)	ca. 500	10
(N)	*Oligia bridghami* Grote & Robinson	Z11-16:Ac + Z11-16:OH (500:1)	ca. 500	> 10
(N)	*Oligia mactata* Guenée	Z11-16:Ac + Z11-16:OH (500:1)	ca. 500	> 10
(N)	*Oncocnemis piffardi* Walker	Z9-14:Ac + Z7-12:Ac (500:1)	100–200	5
	Orthosia hibisci Guenée	Z9-14:Ald + Z11-14:Ald (100:1)	500–1000	3
	Paradiarsia littoralis Packard	Z7-12:Ac + Z5-12:Ac (100:1)	100–200	7
(N)	*Polia assimilis* Morrison	Z11-14:Ac + Z9-14:Ac (100:1)	ca. 1000	1000
				50
	Polia atlantica Grote	Z11-16:Ac + Z11-16:Ald (500:1)	ca. 2000	> 100
(N)	*Polia ingravis* Smith	Z9-14:Ac + Z11-16:Ac (100:1)	ca. 500	> 10
	Polia tacoma Strecker	Z9-14:Ac + Z9-14:Ald (100:1)	1000–2000	> 100
	Scotogramma farnhami Grote	Z11-16:Ald + Z9-14:Ald + Z9-16:Ald (100:1:1)	1000–2000	5
	Trichoplusia ni Hübner	Z7-12:Ac* + Z7-14:Ac (200:1)	500–1000	2

[a] Ratio of captures by trap lures with:without trace components.

Table 2. Effect of sequential addition of trace co-attractants on cone trapping of *Euxoa ochrogaster* males (18 nights)

Co-attractants in lure (optimized amounts)				Males captured, $\bar{X}$/trap[a]	
200 µg	2 µg	0.5 µg	0.5 µg	Test A	Test B
Z5-12:Ac				1.0 c	1.0 d
Z5-12:Ac +	Z7-12:Ac			16.3 b	51.7 c
Z5-12:Ac +	Z7-12:Ac +		Z5-10:Ac	58.0 a	100.0 b
Z5-12:Ac +	Z7-12:Ac +	Z9-12:Ac		52.7 a	no test
Z5-12:Ac +	Z7-12:Ac +	Z9-12:Ac +	Z5-10:Ac	no test	370.7 a

[a] Each test 3 times replicated. Common letters in each test follow numbers not different ($p = 0.05$) by Duncan's multiple range test.

trace components in each blend enhanced lure specificity and increased trap catches many fold (table 1). Greatest trapping increases were noted with *A. indela, H. infixa, Polia tacoma* and *P. atlantica* which could not be trapped significantly with pure main components as test lures, although for trapping, these components were essential in the blends. For other species such as *A. placida, A. venerabilis, E. occulta, O. hibisci* and *T. ni,* trace components enhanced trapping by only a factor of 2–4. The examples of *S. farnhami* and *E. ochrogaster* demonstrate that more than one trace component may be required for a maximally-attractive lure blend. The extremely small amount (0.01%) of trace co-attractant needed for optimal capture of *Abagrotis placida* – 0.1% was strongly inhibitory – suggests the possibility of ultra-high ratios in sex pheromones.

At least for *E. ochrogaster* the various co-attractants are related hierarchically. Presence of both Z5-12:Ac and Z7-12:Ac in lures was essential for attraction; pure Z5-12:Ac was unattractive[6]. Either Z9-12:Ac or Z5-10:Ac, when added to this mixture independently augmented the catch (table 2). A very poor lure was obtained when Z7-12:Ac was omitted from the 4-component blend. This suggests that the presence of Z7-12:Ac as well as Z5-12:Ac is required before Z9-12:Ac or Z5-10:Ac can exert behavioral effects, or that Z7-12:Ac is responsible in this species for particular intermediate behavioral responses between flight initiation and post-landing stages. However, the ethological roles of trace co-attractants have generally not been determined. Capture data (table 2) showing the levels of cone trapping of *E. ochrogaster* males achieved using lures of

Table 3. Effect of variation in trace component levels on cone-trapping of males of *Polia atlantica* and *Euxoa flavicollis.* (10 nights)

Trace component in lure (%)	Males captured, $\bar{X}$/trap[a]	
	P. atlantica[b]	*E. flavicollis*[c]
0	0.0c	1.7b
0.015	no test	11.0a
0.05	11.3b	8.3a
0.15	22.3a	9.0a
0.5	19.0a	8.3a
1.5	1.7c	2.0b
5.0	0.0c	0.0b

[a] Each test 3 times replicated. Common letters in each test follow values not different ($p = 0.05$) by Duncan's multiple-range test. [b] Principal lure compound: Z11-16:Ac, 2000 µg/trap; trace component: Z11-16:Ald. [c] Principal lure compound Z9-14:Ac, 200 µg/trap; trace component Z7-12:Ac.

varying complexity, illustrate the importance of multiple trace constituents in lures, a point recently made in another report[7].

One striking structural feature of all trace components so far known in lepidopterous lures is their direct relation to major components of the lure. In all cases the trace chemicals are 1. homologs, or 2. positional double-bond isomers, or 3. functional group analogs of one or more of the major components; the examples include trace aldehydes or alcohols with acetate major components, and trace alcohol with aldehyde *(Ipimorpha pleonectusa)*. If general, this phenomenon could assist searches for improved insect lures by circumscribing the possible range of trace components which might be involved. Table 3 shows the narrow range of content over which trace co-attractants may function in a lure blend, and illustrates the importance of field-testing at several low levels of addition of trace materials.

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Natural and experimental interspecific hybridization between populations of *Dolichopoda* cave crickets[1]

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Summary. Evidence of hybridization between 2 pairs of allopatric *Dolichopoda* species is provided by electrophoretic analysis. Occurrence of hybrids was revealed by laboratory crosses and in nature both by occasional co-existence due to passive dispersal and by transplantation experiments.

It is usually difficult to provide evidence of hybridization between related cavernicolous species, because in most cases they are allopatric. Only occasionally can this phenomenon be observed as the result of natural or experimentally produced colonization in already inhabited caves. In this paper we present data on the occurrence of hybridization between species of *Dolichopoda* cave crickets, obtained both from morphological analysis and electrophoretic study of enzyme loci.

Figure 1 illustrates the range of the *Dolichopoda* species in Central Italy: *D. schiavazzii, D. baccettii, D. aegilion, D. laetitiae, D. geniculata* and *D. capreensis.* On a morphological basis these species were assigned to 3 subgenera: *Chopardina,* including *D. schiavazzii; Capraiacris,* including *D. baccettii* and *D. aegilion;* and *Dolichopoda,* including *D. laetitiae, D. geniculata,* and *D. capreensis*[2,3]. On the other hand, a recent study of biochemical similarity and the resulting dendrogram based on genetic distance at 15 loci shows 2 major clusters: one including *D. schiavazzii, D. baccettii* and *D. aegilion,* and the other including *D. laetitiae* and *D. geniculata*[4].

In order to test the possibility of hybridization between *Dolichopoda* species, a release experiment was carried out by introducing a sample of *D. laetitiae* into a cave already

Table 1. Numbers and frequencies of hybrids between *D. geniculata* and *D. laetitiae* at Valmarino Cave, after introducing *D. laetitiae* on May 26, 1977

Date of sampling	No. of specimens scored			Relative frequency of hybrids
	D. geniculata	Hybrids	*D. laetitiae*	
15-9-77	24	–	–	–
19-12-77	70	–	1	–
20-3-78	40	1	–	0.024
6-4-78	15	1	–	0.063
19-7-78	9	–	–	–
16-2-79	62	3	4*	0.043
5-6-79	20	–	–	–
21-11-79	23	2	–	0.080
4-3-80	27	2	–	0.069
23-6-80	36	2	–	0.053

* F_1 specimens, resulting from homogamic crosses between introduced *Dolichopoda laetitiae* individuals.