Quasisynergism as Evolutionary Advance to Increase Repellency of Beetle Defensive Secretions

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Phylogenetically derived species of Oxytelinae rove beetles have been found to manufacture definite ratios of different lactone-alkene-solvents in their quinoic defensive secretions. At a certain ratio of solvents the formulation shows a maximal repellency which has actually been found both in naturally occurring beetle secretions and in synthetic mixtures. This phenomenon was identified as quasisynergism since the found ratio of different solvents correlates with a maximal penetration rate of the toxic *p*-toluquinone of the secretions through the integument of an aggressing arthropod.

As a rule defensive secretions of arthropods represent mixtures of different components [1]. However, the biological significance for this chemical complexity is not yet understood. Rove beetles of the Oxytelinae subfamily have been found to sequester the toxic *p*-toluquinone as active principle of their defensive secretions [2–4]. During evolution of Oxytelinae species the solvents for this toxic quinone were changed drastically in order to improve the repellency of the defensive secretion [4].

Here we report for the first time that phylogenetically advanced Oxytelinae species synthesize a mixture of definite volumes of two solvents which considerably increases repellency of the secretion both in naturally occurring and synthetic mixtures if a definite solvent ratio is maintained.

The two solvents in a definite volume ratio have been found to act as quasisynergists by increasing the cuticle permeability for the toxic quinone which then may rapidly permeate the integument of an aggressor.

Members of the rove beetle subfamily Oxytelinae possess paired defensive glands situated within their abdominal tips (Fig. 1A). Quinoic defensive secretion is produced by glandular cells (Fig. 1B, gl) and is transported through a lengthened efferent duct (ed) into a voluminous gland reservoir (res). To repel predators on molestation small droplets of the defensive secretion are depleted from gland reservoirs through an opening (Fig. 1B, op) which is situated on the subdivided ninth tergit of the beetle.

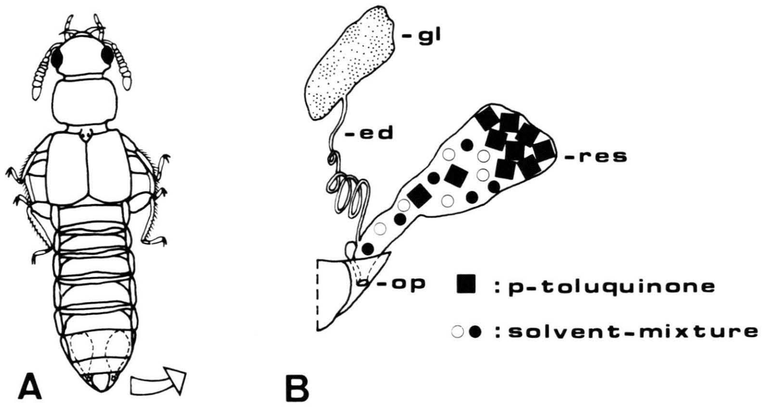

Fig. 1. Dorsal view of the derived rove beetle species *Anotylus sculpturatus* (Oxytelinae, A) showing the enlarged abdominal tip (B) with adhering parts of the defensive gland system (gl = glandular tissue, ed = efferent duct, res = reservoir, op = opening of the gland reservoir). The gland reservoir contains the toxic *p*-toluquinone (■) and a mixture of two solvents (○, ●).

By retaining the active quinoic principle Oxytelinae beetles evolved different types of solvents and obviously increased the defensive value and repellency of the secretion (Fig. 2) by a mere change of the formulation for the active compound [4]. Most primitive Oxytelinae beetle species of the genera *Deleaster* [4] and *Coprophilus* [3] use homologous series of isopropyl- and sec-butyl-esters (Fig. 2) with a minor defensive activity when saturated with quinone. Members of the genus *Syntomium,* however, replace esters by alkenes for solving quinone which results in an increased efficiency of the defensive secretion [5]. Highly advanced Oxytelinae beetle species, however, utilize mixtures of diverse solvents

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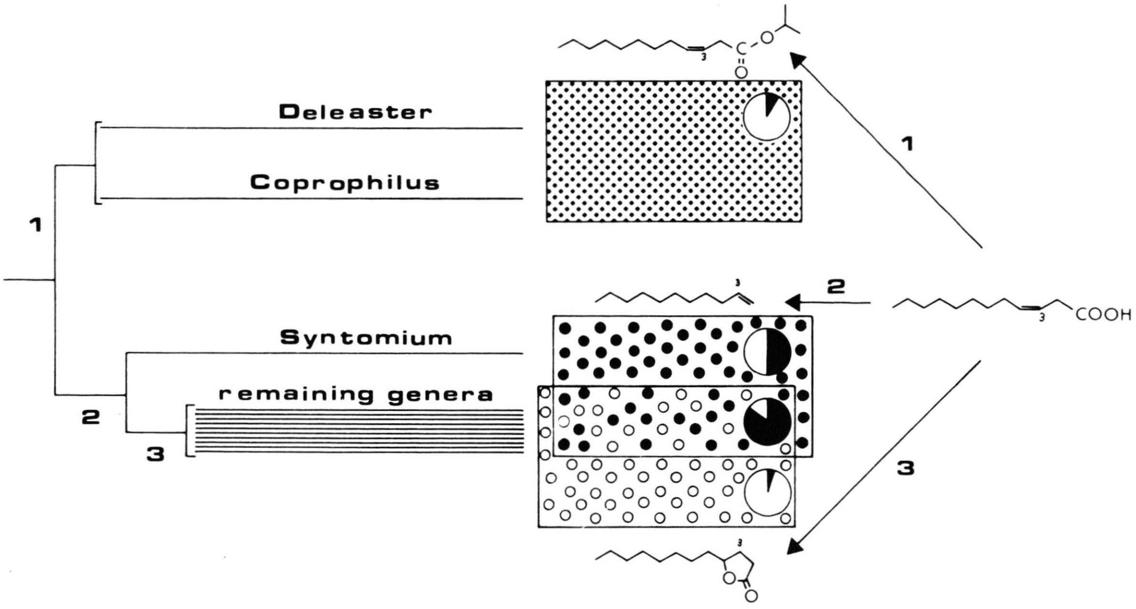

Fig. 2. Evolution of solvent types (center) in the defensive secretions of Oxytelinae beetles. A phylogenetic tree of Oxytelinae (left) is compared with a biogenetic scheme (right) symbolizing the biogenetic steps: isopropylesters (**1**), 1-alkenes (**2**) and γ-lactones (**3**) from a 3-enoic acid precursor. Solid sectors (center) indicate % mortalities of *Lucilia*-larvae ($n = 20$) three hours after having been topically treated with 1 μl quinone saturated synthetic defensive secretion.

composed of a γ-lactone and an 1-alkene [2] and now show a maximal defensive activity. All solvent types found in Oxytelinae beetles are probably biogenetically derived from a single 3-enoic acid [4] which may be either esterified (Fig. 2-**1**) as in *Deleaster* and *Coprophilus* or decarboxylated to produce 1-alkenes (Fig. 2-**2**) as in the remaining Oxytelinae beetles. Pentacyclic γ-lactones of the remaining derived taxa obviously are generated from a hydroxylated 3-enoic acid (Fig. 2-**3**).

It is known that advanced Oxytelinae species such as members of the dung inhabiting genus *Platystethus* may repel dipteran larvae from their brood chambers with minute droplets of their quinoid lactone-alkene defensive secretions [6]. In order to quantify repellencies now the quickness of constriction of *Calliphora*-larvae (Fig. 3C) after treatment with quinone saturated mixtures of different solvent ratios was measured. Not any constriction at all was found by using either pure 1-undecene respectively pure γ-dodecalactone or quinoic γ-dodecalactone (Fig. 3C). This is astonishing since the quinone-saturated γ-lactone distinctly contains more active substance (3.3 mg *p*-toluquinone/ml) than the quinoid 1:5 mixture (ratio lactone:alkene, v:v; 0.28 mg *p*-toluquinone/ml) which exhibits optimal repellency and a minute standard deviation (Fig. 3C). Additional tests with both, lepidopteran (*Spodoptera littoralis* Bois.) and coleopteran larvae (*Tenebrio molitor* L.) reveal that the optimation of repellency of quinoid secretions towards a preponderance of 1-alkenes against lactone seems of general validity as different target arthropods are concerned. Fig. 3A demonstrates that optimal repellency of the 1:5 quinoid defensive mixture primarily results from an optimal increase of *Calliphora* cuticle permeability for the quinoic active principle which is much more evident since the 1:5 mixture contains only $\frac{1}{12}$ of the *p*-toluquinone maximally found to be dissolved within the γ-dodecalactone. Therefore this is a quasisynergistic effect [7] and both solvents certainly do not represent synergists which usually interfere with the *in vivo* detoxication of the quinone. However, similar phenomenons are known from insecticides whose penetration through cuticles and whose effectiveness may also be dependent from the solvents used in the insecticide formulation [8]. Actual solvent ratios for single beetle specimens (Fig. 3B) were only obtained

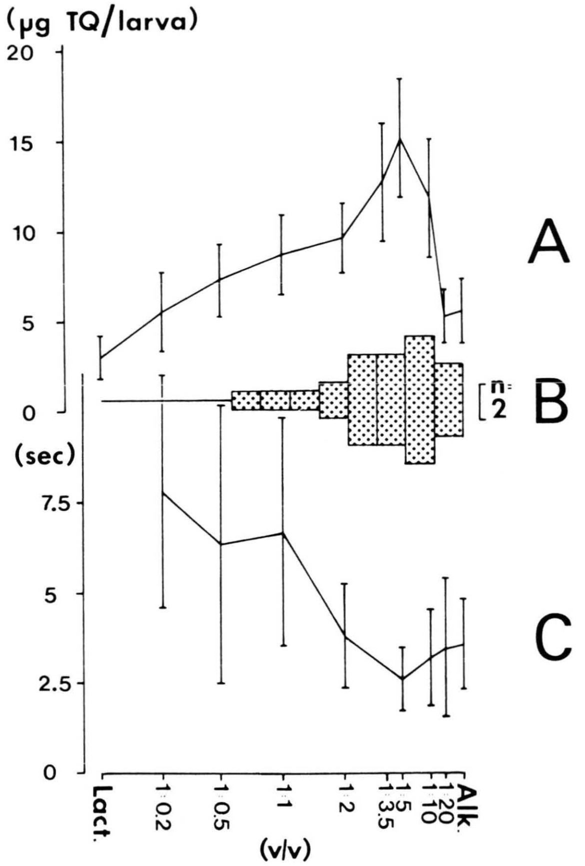

Fig. 3. Quasisynergistic effects by variation of solvent ratio γ-dodecalactone (Lact.) to 1-undecene (Alk.) (v/v; *x*-axis), mixture always saturated with *p*-toluquinone (TQ). A. Penetrated TQ through entire integument into a third stage *Calliphora*-larva ($\bar{x} \pm$ S.D.; $n = 10$) which was immersed for three minutes within definite quinone-saturated mixtures as indicated on *x*-axis. B. Actual ratios of the main components γ-dodecalactone : 1-undecene in the defensive secretions from 26 freshly killed specimens of nine phylogenetically advanced species of the genera *Ancyrophorus, Platystethus, Bledius* and *Oxytelus*. C. Time interval between a topical treatment of a third stage *Calliphora*-larva with 2 µl secretion of the synthetic quinone-saturated mixtures and constriction of the larva ($\bar{x} \pm$ S.D.; $n = 20$).

from freshly killed animals whose gland reservoirs were dissected on ice. Due to gently warming or storing in the refrigerator the ratio of γ-lactone to 1-alkene within the gland shifts to the left side (*x*-axis Fig. 3) due to increased volatility of 1-undecene as compared with the γ-lactone. Volatility tests of different quinoid mixtures additionally showed that a small fraction of lactone in admixture with alkene resulted in a drastic reduction of volatility. Therefore, the γ-lactone acts as fixative for the more volatile 1-alkene. The mean ratio of 1:2.4 which is found in natural defensive secretions of 26 specimens (Fig. 2B) of derived Oxytelinae species therefore may be not only governed by optimal cuticle permeability rates but by other parameters as volatility, too. Although there had been given few reports dealing with the possible advantage of defensive mixtures composed of two fluids (one fluid: active principle) and speculations on cuticle permeabilities [9], this is the first report dealing with the most complicated quasisynergistic allomone system known till now which is composed of two physico-chemically different fluids saturated with a solid as active principle.

It is fascinating that phylogeny corresponds with this picture of a gradually refinement of an allomone system by changing the formulation of the active quinoic principle: Isopropyl- and sec-butylesters of primitive Oxytelinae beetle genera such as *Deleaster* and *Coprophilus* are characterized by minute quinone-penetration rates (penetration rate of isopropyltetradecanoate saturated with *p*-toluquinone: 4.1 ± 1.2 µg TQ/*Calliphora* larva), low mortalities (Fig. 2) and low repellencies (mean constriction time of a *Calliphora*-larva topically treated with quinoic isopropyltetradecanoate: 6.9 ± 3.7 sec) in spite of good wettability characteristics [2]. Even if present as mixture these physico-chemically similar solvents neither show any optimation of repellency nor drastic volatility variations as observed in alkene-lactone-mixtures. The more advanced species of the genus *Syntomium* replaces esters by alkenes and by this way increases quinoic permeability, mortality (Fig. 2) and repellency (as 1-undecene in Fig. 3A, C). As a true anagenesis on a molecular level phylogenetically advanced species manufacture γ-lactones in addition to alkenes the first of which show no effect at all when administered alone but represent highly active formulation principles in admixture (see Fig. 2).

These results indicate that the "raison d'être" of naturally occurring defensive mixtures primarily may be due to quasisynergistic effects caused by the presence of physico-chemically different types of solvents. Therefore a biogenetic need for manufacturing a certain ratio of different solvents is not only favourable within intraspecifically acting pheromone systems to receive species integrity, but is inevitably necessary for allomones too which are directed against a broad array of target organisms. There is an urgent need for both quantitative analyses of defensive mixtures of other arthropods and bioassays in order to test the general validity of this quasisynergistic effect as optimation trend in the evolutionary process of allomone systems.

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