Gel-electrophoretic description of European populations of *Terellia virens* (Loew) (Diptera, Tephritidae); implications for its use as an agent for the biological control of *Centaurea* spp. (Asteraceae) in North America

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Abstract. Allozyme frequencies of 15 enzyme loci, 14 of which were polymorphic, were used to characterize seven *Terellia virens* populations originating from three allopatrically distributed *Centaurea* species. The two populations whose origins were geographically furthest apart, from Israel (on *C. iberica*) and from Switzerland (on *C. vallesiaca*), showed relatively high values of genetic distance from the 5 populations sampled in Austria and Hungary (on *C. maculosa*) (Nei's $D > 0.07$). The latter five displayed a high degree of genetic similarity. No diagnostic (fixed) allelic differences were observed between these three groups of *T. virens* populations, but they could be well characterized by significant differences in allelic frequencies at 9 enzyme loci. Independently of this study, the populations from Switzerland (*C. vallesiaca*) and eastern Austria (*C. maculosa*) were selected as potential source populations for future introductions into North America for the biological control of introduced *C. maculosa* and *C. diffusa*. Based on the observed genetic differences and results from field experiments on the host specificity of these two potential source populations, it is argued that host specificity screening tests should be conducted separately for local (host plant) populations, as such populations might accept a different set of hosts. Biotype mismatch and the risk of spill-overs to native species could thus possibly be reduced.

Key words. Terellia; *Centaurea*; allozymes; host races; biological control.

Introduction

The seed head fly *Terellia virens* (Loew) (Dip.: Tephritidae), which has been reported to feed on 14 different *Centaurea* (Asteraceae) species [1], was studied at the European Station of the Commonwealth Institute of Biological Control (CIBC), Switzerland, between 1986–1989, in order to investigate its potential as a biological control agent for *Centaurea maculosa* Lam. and *C. diffusa* Lam. in North America [2].

In biological control projects against weeds, the studies predicting an agent's potential host range and its impact on weed density are of critical importance. Experimental investigations of the host range of a potential control agent to guarantee its relative 'safety' are especially important in classical biological control projects, which attempt to control an alien weed by introducing the weed's natural enemies from its area of origin [3]. However, dietary specialization is often a flexible attribute of a population in response to features of its particular community, rather than an attribute of a species throughout its geographical range [4]. The coupling of mating site, oviposition site and larval food resources is a property of many specialized herbivorous insects. In such a case, the switch to a new host plant may simultaneously channel the gene flow and completely change the food and habitat niche, possibly leading to the development of host races [5–12]. The formation of host races as a result of either allopatric or sympatric processes is considered to be a widespread phenomenon in tephritids [5], and recent studies reported significant differences in allele frequencies between fly populations reared from their sympatric host plant species [10–12].

Unruh and Goeden [8] also reported high levels of genetic and behavioural differentiation between *Carduus* and *Silybum* races of the weevil *Rhinocyllus conicus*, introduced into California for the biological control of composite thistles. They could further show, on the basis of allozyme frequency data, that the weevil populations recently found on two thistle species native to California probably all originate from one race only. Genetic evaluations of a widespread complex of single species populations should therefore be performed prior to host-screening tests in order to assess the degree to which a candidate

species of biological control agent is differentiated along geographic and host plant lines.

So far, genetic aspects of agent selection and release strategies have not been considered in biological control projects[3, 13, 14]. Genetic information about source populations prior to release is generally lacking, so that no general recommendations can yet be proposed to biological control workers[15, 16]. The aim of this study was to establish an 'identity-card', using electrophoretic markers, for *T. virens* populations that might be used as source populations for future introductions into North America. We further wish to provide a basis for later comparisons with released and established populations, which will allow various genetic hypotheses to be tested in the future.

The species

The holarctic genus *Terellia* consists of about 30 species belonging to 9 species groups[17]. The three species of the *T. virens* group, *T. virens* (Loew), *T. zerovae* Korneyev and *T. uncinata* White occur only in the western and central Palaearctic.

Terellia flies attack the capitula of plant species of the tribe Cardueae (= Cynareae) of the family Asteraceae, but the three species of the *T. virens* group are associated with *Centaurea* species only. Table 1 lists the geographical regions and host plants of the species *T. virens* from literature[1] and field records[18]. Extended screening, comprising oviposition preference and larval development tests with 57 plant species, was carried out at the CIBC to ensure that the degree of host specificity was that required for an approved biological control agent[2]. The results were in agreement with the field and literature records and confirmed the narrow host range of *T. virens* and its preference for species in the *Centaurea* subgenus *Acrolophus* (table 1). *Terellia virens* attacks young opening flowers and reaches infestation rates of up to 60% of the flower heads with a slightly aggregated, density-independent distribution. Depending on climatic conditions, a partial second summer generation can occur[18].

Table 1. Host plant associations and geographic distribution of *T. virens*

Host plant[a]	Record[b]	Country[c]
Centaurea		
Subgenus *Acrolophus*		
C. arenaria	F	RO
C. diffusa	F	RO, SU
C. leucophaea	F	F
C. spinabadia (as *C. coerulescens*)	L	Med.
C. paniculata	L	Med.
C. macuosa	F/L	A, H, F, RO/CS, D
C. vallesiaca	F	CH
Subgenus *Calcitrapa*		
C. hyalolepis	L	IL
C. iberica	F/L	IL/JOR
C. pallescens	L	IL, JOR
Subgenus *Jacea*		
C. nigrescens	L	FRG
C. pectinata	L	F
Subgenus *Phalolepis*		
C. alba	L	I
Subgenus *Seridia*		
C. aspera	L	F
Subgenus *Solstitiaria*		
C. melitensis	L	Med.

[a] Subgeneric classification follows Dostal[21]. [b] F: field record (Marquardt[18]); L: literature record in White[1]. [c] A: Austria; CH: Switzerland; CS: Czechoslovakia; FRG: West Germany; F: France, H. Hungary; I: Italy; JOR: Jordan; IL: Israel; Med: Mediterranean region; RO: Romania; SU: Soviet Union.

The genus *Centaurea* consists of around 500 species of predominantly Mediterranean distribution. Three host species were considered in this study: 1) The eurasiatic species *C. maculosa*, which occurs in eastern Europe in continental xeric plant associations, but reaches its highest local densities in its western distribution area at sites disturbed by human activities, such as roadsides or abandoned or overgrazed pastures[19]. 2) The taxonomically closely related *C. vallesiaca* Jordan, which probably occurs only in the Western Alps[20] and 3) *C. iberica* Trev., which is limited to south-eastern Europe[21].

All three species of the *T. virens* group are potential biological control agents for *C. maculosa* and the closely related east-European *C. diffusa* (cf. table 1). Both *Centaurea* species were introduced into North America at the turn of this century, and have since become noxious rangeland weeds of great economic importance[22].

Material and methods

Collection. Seven populations of the species *T. virens* – originating from three host plants and from localities ranging from Switzerland to Israel – were investigated in this study (table 2). The distribution areas of the three host plant species are geographically separated, and no alternative host plants could be found at the sites studied. A minimum of 500 flower heads were collected at random at each site during August and September 1985. The flower heads were kept at 18–20 °C until the following autumn, except from early October to early April when they were overwintered at 5 °C. The majority of the flies emerged in May and June 1986. Flies from Israel originated from flower heads collected in autumn 1986, and emerged in June 1987. Only sites where no other *Terellia* species occurred (especially no other species of the *virens* group; I. White, pers. communication), were used.

Table 2. Origin, host plant and number of *T. virens* analysed

Site	Code	N	Country[a]	Region	Host plant
Brig	VS	60	CH	Valais	*C. vallesiaca*
Sollenau	SO	48	A	Lower Austria	*C. maculosa*
Hornstein	HO	73	A	Burgenland	*C. maculosa*
Velence	VE	82	H	Western-H	*C. maculosa*
Julia Major	JM	83	H	Central-H	*C. maculosa*
Pilis	PI	60	H	Eastern-H	*C. maculosa*
Mont Hermon	IL	100	IL	Northern-IL	*C. iberica*

[a] cf. table 1 for reference to country codes.

Table 3. Summary of allele frequencies in seven populations of *T. virens* at the 14 polymorphic enzyme loci (see table 2 for references to host plant, country and population codes).

	Host-Plant	CV	Centaurea maculosa					CI
	Country	CH	Austria	Austria	W-Hung.	C-Hung.	E-Hung.	ISRAEL
LOCUS	Alleles	VS	SO	HO	VE	JM	PI	IL
PGI	N	18	12	36	49	43	23	36
	59		0.04	0.01	0.04		0.04	0.03
CA*	70	0.50	0.50	0.46	0.43	0.26	0.24	0.41
tris-glyc.	90		0.04	0.01	0.02		0.02	0.03
	100	0.50	0.42	0.51	0.51	0.74	0.70	0.53
GPD	N	15	10	30	52	48	17	50
	100	0.80	0.50	0.50	0.50	0.38	0.50	0.50
CA	115					0.10		
tris-glyc.	128	0.20	0.50	0.50	0.50	0.42	0.50	0.50
	143					0.10		
GOT	N	22	17	31	47	33	17	42
	48				0.01			
starch	56							0.01
TCB	72	0.02	0.06	0.02	0.02	0.05	0.13	0.06
	84	0.05						0.01
	100	0.87	0.91	0.98	0.95	0.92	0.87	0.90
	106	0.04						
	119	0.02			0.01	0.03		
	138		0.03					0.01
HK	N	21	6	20	53	47	17	51
	86							0.01
CA	89				0.01			0.09
tris-glyc.	93			0.14	0.03	0.04	0.12	0.24
	96	0.04		0.16	0.19	0.12		0.04
	100	0.66	0.67	0.50	0.59	0.63	0.74	0.50
	103	0.14		0.10	0.06	0.06	0.03	0.02
	105	0.16	0.33	0.1	0.12	0.14	0.12	0.1
MPI	N	15	10	24	55	36	22	50
	66		0.05			0.01		
CA	76		0.15	0.10	0.05	0.03	0.02	0.02
tris-glyc.	86	0.37	0.45	0.27	0.31	0.27	0.34	0.42
	100	0.50	0.35	0.44	0.47	0.53	0.50	0.51
	113	0.10		0.19	0.16	0.16	0.14	0.05
	118	0.03						
ACON-1	N	60	45	61	76	43	60	77
CA	81			0.02	0.05			
tris-glyc.	89	0.25	0.23	0.20	0.22	0.20	0.26	0.05
and	100	0.73	0.61	0.71	0.65	0.70	0.61	0.86
starch	107	0.02	0.16	0.04	0.08	0.09	0.11	0.08
TcB	116			0.02			0.02	
ACON-2	N	21	11	26	57	49	24	46
	64	0.48						
cf. ACON-1	100	0.52	1.00	1.00	0.99	0.99	0.98	0.95
	150				0.01	0.01	0.02	0.05
PGM	N	22	12	41	63	50	23	53
	60	0.02						0.01
starch	100	0.82	0.46	0.38	0.41	0.45	0.33	0.81
IV	152		0.25	0.08	0.04	0.13	0.04	0.12
	200	0.16	0.25	0.49	0.49	0.32	0.50	0.06
	258		0.04	0.05	0.05	0.08	0.13	
	313				0.01			
	420					0.01		
MDH-1	N	49	45	57	82	83	55	100
	51		0.03	0.03	0.01	0.02	0.03	0.03
starch	71				0.01			
ACPH	85			0.01				
	100	0.96	0.94	0.93	0.96	0.95	0.93	0.95
	125			0.01				
	129	0.04	0.02	0.03	0.01	0.03	0.03	
	136						0.01	0.02
MDH-2	N	20	10	60	65	58	22	55
	60			0.01	0.02	0.02		
starch	100	1.00	1.00	0.98	0.94	0.94	0.98	1.00
ACPH	112			0.01	0.03	0.03	0.02	
	125				0.01	0.01		
ICD-1	N	56	48	73	76	47	40	91
	82	0.13	0.04	0.01	0.05	0.02	0.06	0.02
starch	93				0.01	0.01	0.03	
ACPH	100	0.87	0.96	0.99	0.94	0.97	0.91	0.98
ICD-2	N	15	10	50	53	47	19	49
	79			0.01	0.01	0.00	0.00	0.01
starch	86		0.10		0.07	0.02	0.18	0.01
ACPH	100	1.00	0.90	0.99	0.92	0.98	0.82	0.97
PGD	N	22	9	34	58	47	18	48
	63		0.33		0.05	0.09	0.03	0.04
starch	100	1.00	0.67	0.99	0.91	0.87	0.94	0.88
ACPH	134			0.01	0.02	0.02		0.03
	188				0.02	0.02	0.03	0.05
PEP	N	20	9	44	48	34	20	51
	93			0.02				
starch	100	0.02	0.50	0.34	0.25	0.36	0.23	0.92
ACPH	105	0.05		0.02	0.08	0.08	0.07	0.04
	111	0.15		0.05	0.04	0.19	0.10	0.02
	155	0.78	0.50	0.57	0.62	0.37	0.60	0.02
	HL mean	0.32	0.36	0.34	0.35	0.27	0.33	0.29
	SE	0.08	0.09	0.08	0.08	0.07	0.08	0.08
	A	2.47	2.40	3.13	3.60	3.33	3.07	3.21
	P	0.73	0.80	0.87	0.93	0.93	0.93	0.87

HL mean: Mean observed heterozygosity per locus; A: Average number of alleles per locus; P: Proportion of polymorphic loci; HL mean, A and P are based on 15 enzyme loci.

* see text for references to support material and buffer.

Freshly emerged adults were stored in liquid nitrogen until electrophoretic analysis was performed.

Electrophoresis. Standard horizontal starch-gel electrophoresis techniques[23] were used to test 22 enzyme systems with 9 different buffer systems in order to optimize resolution and separation. In addition, loci for which the results were unsatisfactory were also checked with cellulose-acetate (CA)-gels[24]. Whole flies were ground in 15 µl of distilled water. For the CA-plates 3 µl of the homogenate was taken out and diluted with 5 µl of distilled water. Tris-glycine was used both to soak the plates and as electrode buffer. For starch gels, 2 µl of bromophenol-blue was added to the remaining homogenate to mark the running front. Paper wicks (3 × 10 mm) were saturated with the homogenate and inserted into the gels. 20 flies were applied on each starch gel (13 × 20 cm) and CA-plate (76 × 76 mm, with 2 starting lines). An applicator was used to load the CA-plates. Based on resolution and separation properties, 12 enzyme systems resulting in 15 loci were finally selected for scoring (cf. Harris and Hopkinson[25] for nomenclature): aconitase (ACON-1 and ACON-2), oxaloacetate transferase (GOT), glycerol-3-phosphate dehydrogenase (GPDH), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), hexokinase (HK), isocitrate dehydrogenase (IDH-1 and IDH-2), malate dehydrogenase (MDH-1 and MDH-2), mannose phosphate isomerase (MPI), 6-phospho-gluconate dehydrogenase (6-PGD), peptidase (PEP-1), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM). Three buffers were used for the starch gels; IV: tris/citrate (gel: 0.008/0.003 molar, pH 6.7; electrodes: 0.22/0.09 molar, pH 6.3); TCB: tris/citrate (gel: 0.076/0.004 molar, pH 8.7; electrodes: 0.34/0.08 molar, pH 8.0) and ACPH: tris-Na-citrate/Na-dihydrogenphosphate (gel: 0.11/0.24 molar, pH 6.7; electrodes: gel 1:40), and tri-glycine (0.25/1.92 molar, pH 8.5) for the CA-gels (cf. table 3 for combinations).

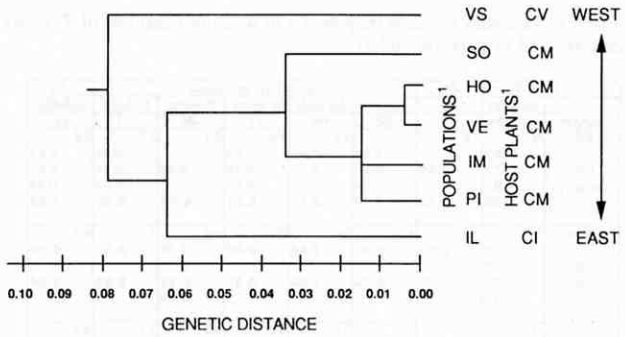
A total of 506 flies were analysed. Designation of alleles is based on mobility of the electromorphs and indicates the differences in relative mobility of the most frequent electromorph of each enzyme relative to the population from Israel (index 100). Allele frequencies were used to test differences between sites based on single loci by means of the minimum discrimination statistics 2-$\hat{I}$[6, 26]

and to calculate Nei's coefficients of genetic identity and distance[27]. The latter were used to produce a dendrogram with the UPGMA clustering algorithm[28].

Results

Of the 15 scorable loci, only the G3PDH locus was monomorphic, with the same allele for all populations. The number of alleles varied between 3 and 8 alleles for the remaining 14 loci (table 3). Allele assignment of the electromorphs is supported by the fact that genotypic frequencies only rarely deviated from a Hardy-Weinberg equilibrium.

The genetic distance values ranged from 0.04 between the central European populations from Hornstein and Velence to 0.118 between the geographically most distant populations from Switzerland (CH) and Israel (IL) (table 4). The phenogram constructed from the matrix of genetic distances (UPGMA-method) shows the very close relationship between the Austrian and Hungarian populations, which cluster in the dendrogram at a level of $D < 0.015$, except that from Sollenau, which clustered at $D = 0.034$ (fig.). The two populations from Switzerland and Israel, which are geographically furthest apart and which each originate from a different host plant (table 2) different from that of the central European populations (*C. maculosa*), are also separated in the dendrogram,



Phenogram based on UPGMA clustering of a matrix of Nei's genetic distances, derived from allelic frequencies at 14 polymorphic loci in seven *T. virens* populations. 1) cf. table 2 for reference to population and host plant codes.

displaying genetic distance values of $D = 0.079$ and $D = 0.064$, respectively.

Significant differences between the seven populations were found at 9 enyzme loci, but only 6 loci differed significantly between the central European *C. maculosa* populations, confirming the distinct position of the CH- and IL-populations (table 5).

No diagnostic (fixed) allelic differences were detected between the seven populations. Deviations from the allelic frequency of the commonest allele of the reference population IL were most pronounced for the GPD, PGM and PEP loci (table 3). The isolated, most western population from the Swiss Valais (*C. vallesiaca*) can be characterized by the high allelic frequency of 0.80 for the GPD^{100} allele and of 0.78 for the PEP^{155}, and the low frequency of 0.52 for the ACON-2^{100} (the ACON-2^{100} allele was fixed or nearly fixed in all other populations). In addition, and contrasting with all other populations, the ICD-2^{100} and the PGD^{100} alleles were fixed only in the VS population. The population from IL – the most eastern sample, originating from *C. iberica* – differs from all other populations mainly in its high frequency of 0.86

Table 4. Genetic identities (above diagonal) and genetical distance (below diagonal) among populations. See table 2 for references to population codes

Population		VS	SO	HO	VE	IM	PI	IL
West	VS		0.917	0.936	0.943	0.929	0.931	0.889
↑	SO	0.086		0.968	0.971	0.966	0.961	0.947
↕	HO	0.066	0.033		0.996	0.983	0.982	0.941
↕	VE	0.059	0.029	0.004		0.984	0.988	0.930
↕	IM	0.073	0.034	0.017	0.016		0.983	0.951
↓	PI	0.072	0.040	0.018	0.012	0.017		0.918
East	IL	0.118	0.055	0.061	0.073	0.050	0.086	

Table 5. Summary of tests for inhomogeneity (2-Î) for each of the 14 polymorphic enzyme loci between seven populations and among five and four central European populations (degrees of freedom, 2-Î-values and significance levels (p) are given).

Locus	All populations combined Df	Î-value	p	All five *C. maculosa* populations Df	Î-value	p	Four *C. maculosa* populations (excl. SO) Df	Î-value	p
PGI	18	28.04	n.s.	12	24.25	*	9	20.56	*
GPD	18	64.15	***	12	42.75	***	9	20.56	*
GOT	42	13.87	n.s.	16	8.69	n.s.	9	4.23	n.s.
HK	36	93.56	***	20	27.76	n.s.	15	22.59	n.s.
MPI	30	31.83	n.s.	16	17.67	n.s.	12	4.92	n.s.
ACON-1	24	85.84	***	16	26.58	*	12	19.29	n.s.
ACON-2	12	107.35	*	4	0.14	n.s.	3	0.45	n.s.
PGM	36	126.15	***	20	22.03	n.s.	15	15.84	n.s.
MDH-1	36	20.46	n.s.	24	3.87	n.s.	18	5.95	n.s.
MDH-2	18	7.97	n.s.	12	2.99	n.s.	9	4.24	n.s.
ICD-1	12	30.81	**	8	10.97	n.s.	6	9.84	n.s.
ICD-2	12	25.2	*	8	19.87	*	6	19.82	**
PGD	18	33.67	**	12	22.55	*	9	10.59	n.s.
PEP	24	215.82	***	16	31.81	*	12	27.37	**

n.s., not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

for the ACON-1^{100} and of 0.92 for the PEP^{100} allele, as well as for the relatively high frequencies of the slower HK alleles (table 3). The Sollenau population (SO) differs significantly from the other Austrian and Hungarian populations at the ACON-1 and PGD loci (table 5). The remaining central European populations seem to be genetically very similar, as was already documented by their small genetic distances (table 4, fig.).

The average number of alleles per locus (A) detected in this study varied from 2.40 (total of 36 alleles) of the Sollenau population up to 3.60 (total of 54 alleles) of the Velence population. Differences in allele numbers between populations, however, can be well explained by the different number of individuals stained for each locus, explaining 75% of the variation in total number of alleles, displaying a highly significant linear regression ($p < 0.01$). The proportion of polymorphic loci (P), was relatively high for all seven populations, ranging from 0.73 to 0.93. The two populations with the smallest sample number – VS and SO – also displayed the highest number of monomorphic loci (table 3).

Discussion

Genetic distances between the central European *C. maculosa* populations are very small. The highest calculated value of $D = 0.118$ between the most distant VS and IL populations still lies considerably below the value of 0.230 ± 0.016 ($\bar{x} \pm SE$) given by Thorpe[29] to characterize genetic differences between subspecies.

The fact that the two most distant populations also originate from different host plants does not allow the direct analysis of the effect of host plant differences. The maximum geographical distance between the central European populations corresponds to only about one half of the distance between the VS population and the closest *C. maculosa* population, and only 14% of the corresponding distance to the IL population, respectively. Tephritids show in general little electrophoretically detectable genetic differentiation, which may reflect a history of rapid speciation due to strong, host-mediated, sexual selection[30]. The high genetic similarity, mainly among the central European populations, may also be due to the assumed good dispersal ability of the fly, indicated by its wide distribution, which covers three quarters of the host plant's distribution[2].

The observed genetic variability, expressed by values of $P > 0.73$ and $H_L > 0.27$, is relatively high in all populations, as compared with data from electrophoretical studies of other Tephritidae[31] (Lehr, unpublished data). The limited genetic distance between the three groups of *T. virens* populations originating from different host plants (fig.) nevertheless allows a good characterization of the three groups, based on significant differences in allelic frequencies for 9 enzyme loci (table 5).

Independently of this study, the *T. virens* populations from VS and SO were chosen as possible source populations for release in North America, and first introductions into quarantine facilities are planned for 1991 (D. Schroeder, pers. communication). The host specificity of the VS and SO populations was also investigated in a parallel open field experiment with seven closely related *Centaurea* species, including the two host plants *C. maculosa* and *C. vallesiaca* and the North American *C. maculosa* biotype (cf. Müller[32] for biotype differences). Preliminary data analysis showed marked differences between the two populations, both in their acceptance of the North American target weed, and in the number and proportion of the seven *Centaurea* species infested (Marquardt and Müller, in preparation). Hence, the existence of the observed genetic differentiation, together with these differences in host plant specificity, emphasizes the importance of conducting separate host specificity screening tests for each local (host plant) population, as the populations may differ in their preferred hosts[8, 9, 33]. This is especially important in view of the growing concern that introduced biological control agents might jeopardize native endangered species. Such studies are also an important complement to ecoclimatic matching[34] for choosing optimal source populations, as biotype mismatch[35, 36] can be avoided and the risk of spillovers to native species reduced. So far, no screening tests have been deliberately carried out at the level of local (host plant) populations for any insect agent released or considered for the biological controls of weeds (D. Schroeder, pers. communication).

The electrophoretic data presented will also allow the genetic variability of the source population to be related to the rate of establishment and increase of populations, and allelic frequencies can be compared between the source population and the various stages of the introduced population. In particular, various genetic hypotheses could be tested by releasing variable numbers of individuals, and single demes versus 'mixed-genotypes', and by varying the number of releases. This, however, needs a careful design of release strategies, as will be discussed elsewhere[16]. Biological control projects offer a unique and as yet greatly underexploited opportunity to test genetic hypotheses, and this in turn offers the hope that predictions about biological weed control projects will become more accurate.

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