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*Article*

# *Sideritis elica*, a New Species of Lamiaceae from Bulgaria, Revealed by Morphology and Molecular Phylogeny

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**Abstract:** *Sideritis elica*, from the Rhodope Mountains, is described as a species new to science. Results of a detailed morphological analysis were combined with the data of molecular analyses using DNA barcoding as an efficient tool for the genetic, taxonomic identification of plants. The combination of morphological features distinguishes the new species well: Its first three uppermost leaf pairs are significantly shorter and wider, the branchiness of the stems is much more frequent, the whole plant is much more lanate, and it looks almost white, as opposed to the other closed species of section Empedoclia, which look grayish green. The molecular analysis, based on the rbcL and trnH-psbA regions, supports the morphological data about the divergence of *Sideritis scardica* and *Sideritis elica*. The studied populations of the two taxa were found to be genetically distant (up to 6.8% polymorphism for trnH-psbA) with distinct population-specific nucleotide patterns, while no polymorphism in the DNA barcodes was detected within the *Sideritis elica* population. The results confirm the existence of a new species called *Sideritis elica*, which occurs in the nature reserve Chervenata Stena, located in the northern part of the Central Rhodope Mountains. There were only 12 individuals found in the locality, which underlines the necessity of conservation measures.

**Keywords:** medicinal plants; phenotypic variation; cryptic species; taxonomy



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## 1. Introduction

Genus *Sideritis* (Lamiaceae, Lamioideae) comprises more than 150 species distributed in the temperate and tropical areas of the Northern Hemisphere [1,2] and subdivided into two subgenera: *Sideritis* and *Marrubiastrum* (Moench.) Mendoza-Heuer. Southeastern Europe and the Eastern Mediterranean, with about 50 species, represent a center of diversity, particularly of section *Empedoclia* (Rafin.) Bentham of the subgenus *Sideritis*, with 45 species in Turkey [3], and about 10 species in Greece and the Balkans in general [4–6]. The number of species depends on their taxonomic treatment and concepts, which are not straightforward because of the high level of polymorphism, including ecotype diversity and hybridization among the species [7].

In Bulgaria, *Sideritis* is represented by four species, two of them belonging to section *Hesiodia* Bentham (*S. montana* L. and *S. lanata* L.), and two belonging to section *Empedoclia* (*S. scardica* Griseb. and *S. syriaca* L.) [8]. All but *S. montana* are considered rare and endangered species in need of measures for conservation [9–11]. Moreover, *S. scardica* and *S. syriaca* are considered essential medicinal plants and are subject to cultivation. While the two species of section *Hesiodia* are discrete and well-distinguishable, there are still some taxonomic uncertainties within the species of section *Empedoclia* [7].

A typical distribution pattern of *Sideritis* species of section *Empedoclia* is the high percentage of endemism [3,12,13]. *S. scardica* is endemic to the Balkan Peninsula, while *S. syriaca sensu lato* is believed to have wider distribution [4]. Both species naturally occur exclusively on limestone although they can be cultivated in a broader range of soil pHs [14].

**Table 3.** Oligonucleotide primers used for amplification of DNA barcode regions, and the respective PCR conditions.

| Barcode Region | Primers | Primer Sequences 5′-3′ | PCR Conditions |
|---|---|---|---|
| matK | matK-1RKIM-f | ACCCAGTCCATCTGGAAATCTTGGTTC | 95 °C 5 min<br>(95 °C 30 s,<br>51 °C 50 s,<br>72 °C 1 min)—35 cycles<br>72 °C 7 min |
| | matK-3FKIM-r | CGTACAGTACTTTTGTGTTTACGAG | |
| rbcL | rbcLa-F | ATGTCACCACAAACAGAGACTAAAGC | 94 °C 4 min<br>(94 °C 30 s,<br>55 °C 30 s,<br>72 °C 1 min)—35 cycles<br>72 °C 10 min |
| | rbcLajf634R | GAAACGGTCTCTCCAACGCAT | |
| trnH-psbA | trnH-F | CGCGCATGGTGGATTCACAATCC | 94 °C 4 min<br>(94 °C 30 s,<br>55 °C 30 s,<br>72 °C 1 min)—35 cycles<br>72 °C 7 min |
| | psbA3_r | GTTATGCATGAACGTAATGCTC | |
| ITS | ITS F1 | CCTTATCATTTAGAGGAAGGAG | 94 °C 5 min<br>(94 °C 30 s,<br>50 °C 30 s,<br>72 °C 1 min)—35 cycles<br>72 °C 5 min |
| | ITS 4 | TCCTCCGCTTATTGATATGC | |

Candidate DNA barcode sequences for each barcode region were aligned via MEGA-X, and consensus sequences were subjected to further analyses using the software package Geneious. The phylogenetic trees were constructed using the Jukes–Cantor genetic distance model [30], and the UPGMA tree-building method. Evolutionary divergence was tested using the Tamura 3-parameter model [31] implemented in MEGA-X software [32].

Taxonomic assignment of the *Sideritis* specimens was performed through BLAST analyses in Geneious against publicly available accessions in NCBI. The estimates of within population and between population divergence were calculated in MEGA-X [32].

## 5. Conclusions

In this study, we performed a morphological and DNA barcoding analysis of representatives of *Sideritis scardica* populations from two geographically distant floristic regions in Bulgaria. The *Sideritis* population from the reserve Chervenata Stena (CHE) was found to be phenotypically distinct from *Sideritis scardica*. This allowed us to state that the population from the reserve represents a new a new species we called *Sideritis elica* Aneva, Zhelev and Bonchev. The genetic divergence between *S. scardica* and *S. elica* was supported based on rbcL and trnH-psbA markers. The data has two main implications. First, our study implies that eco-geographical and demographic conditions enhance genetic diversification and occasionally the speciation within the genus *Sideritis*. Second, our study highlights the importance of the DNA barcoding method to unravel patterns of genetic variability at species in support of classical morphological approaches. Further studies on a larger set of *Sideritis* populations could give deeper insight into the ecological dynamics of this endemic genus of high medicinal value for Bulgaria, with practical implications for its conservation.

**Author Contributions:** Conceptualization, I.A.; methodology, I.A., P.Z. and G.B.; software, I.A., P.Z. and G.B.; validation, I.A. and P.Z.; formal analysis, I.A., P.Z. and G.B.; investigation, I.A., P.Z. and G.B.; resources, I.A. and P.Z.; data curation, I.A., P.Z. and G.B.; writing—original draft preparation, I.A. and P.Z.; writing—review and editing, I.A., P.Z. and G.B.; visualization, I.A. and P.Z.; supervision, I.A. and P.Z.; project administration, I.A., P.Z. and G.B.; funding acquisition, I.A., P.Z. and G.B. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

# Fingerprinting the genetic variation and intergeneric hybrid dynamics in the family Asteraceae (genera *Helianthus, Echinaceae, Tagetes* and *Verbesina*) using iPBS markers

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**Abstract**
Transposable elements constitute a large fraction of plant genomes and represent a powerful marker tool for genetic diversity studies. Here, the retrotransposon-based marker method inter primer binding sites (iPBS) was used to assess the genetic variation and intergeneric hybrid dynamics in the family Asteraceae by studying genera *Helianthus, Echinaceae, Tagetes, Tithonia* and *Verbesina*. Two selected iPBS primers (2222 and 2224) detected intergeneric polymorphism in the range 44.8% - 93.3% (mean 70%) and 85.7% - 100% (mean 89.5%) respectively. Moreover, iPBS markers allowed the genetic discrimination at within-species level between varieties of *H. annuus* (35.7% and 19.1%) but also between single cross's segregating intergeneric hybrids (28.6% and 40%). The inheritance of iPBS markers and the parental genomes respectively in intergeneric hybrids of *H. annuus* has been manifested by the non-random elimination of markers mainly of origin of wild species and the preferential inheritance of markers unique to *H. annuus*. Such instability evidences genomic reconstruction involving LTR elements. In conclusion, the iPBS method stands as a reliable approach for the evaluation of genetic diversity of Asteraceae germplasms and perspective for use in the breeding practice of sunflower and related species.

**Keywords** Transposable elements · Molecular markers · Sunflower hybrids · Genetic diversity

**Abbreviations**

| | |
|---|---|
| TEs | Transposable Elements |
| IRAP | Inter Retrotransposon Amplified Polymorphism |
| iPBS | inter Primer Binding Sites |
| REMAP | Retrotransposon Microsatellite Amplified Polymorphism |

## Introduction

Wide (intergeneric and interspecific) hybridizations commonly have a great potential for crop improvement by widening the genetic base from which plant breeder can select desirable traits (Liu et al. 2005). In genus *Helianthus*, there has been an increasing interest in the use of wild sunflower relatives - a valuable source of desirable agronomic traits (Breton et al. 2012; Vassilevska-Ivanova et al. 2013, 2014, 2015, 2018; Liu et al. 2017; Seiler et al. 2017). However, most of the wild relative species remain untapped as usable germplasm. The reason for this is that the genus *Helianthus* has no close relatives; the pattern of distributions of phylogenetic markers suggested that wide hybridization is not uncommon within the larger group to which the common sunflower belongs (Seiler et al. 2017).

Transposable elements (TEs) are well suited as molecular markers to monitor natural and stress-induced genetic diversity (Schulman et al. 2004). The reason for this is their ubiquitous distribution in plant genomes (Schnable et al. 2009; Choulet et al. 2014) and susceptibility to activation and transposition in response to stress such as pathogen attacks, wounding, extreme temperature etc. (Wessler 1996; Grandbastien 1998). In this line, intergeneric hybridizions often appears as a „genome shock "capable of triggering changes in gene regulation and chromosome rearrangements (Adams et al. 2003; Paun et al.

**Electronic supplementary material** The online version of this article (https://doi.org/10.2478/s11756-019-00363-3) contains supplementary material, which is available to authorized users.

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## Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by the authors.

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## References


Adams KL, Cronn R, Percifield R, Wendel JF (2003) Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. Proc Natl Acad Sci U S A 100: 4649–4654. https://doi.org/10.1073/pnas.0630618100

Andeden EE, Baloch FS, Derya M, Kilian B, Özkan H (2013) iPBS-Retrotransposons-based genetic diversity and relationship among wild annual *Cicer* species. J Plant Biochem Biotechnol 22:453–466. https://doi.org/10.1007/s13562-012-0175-5

Baloch FS, Alsaleh A, de Miera LES, Hatipoğlu R, Ciftci V, Karakoy T, Yıldız M, Ozkan H (2015a) DNA based iPBS-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from Turkey. Biochem Syst Ecol 61:244–252. https://doi.org/10.1016/j.bse.2015.06.017

Baloch FS, Derya M, Andeden EE, Alsaleh A, Comertpay G, Kilian B, Ozkan H (2015b) Inter-primer binding site retrotransposon and inter-simple sequence repeat diversity among wild *Lens* species. Biochem Syst Ecol 58:162–168. https://doi.org/10.1016/j.bse.2014.12.002

Baranek M, Meszaros M, Sochorova J, Čechova J, Raddova J (2012) Utility of retrotransposon-derived marker systems for differentiation of presumed clones of the apricot cultivar Velkopavlovicka. Sci Hortic 143:1–6. https://doi.org/10.1016/j.scienta.2012.05.022

Barton NH (2001) The role of hybridization in evolution. Mol Ecol 10: 551–568. https://doi.org/10.1046/j.1365-294x.2001.01216.x

Basirnia A, Darvishzadeh R, Abdollahi MB (2014) Retrotransposon insertional polymorphism in sunflower (Helianthus annuus L.) lines revealed by IRAP and REMAP markers, Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana. https://doi.org/10.1080/11263504.2014.970595

Breton C, Gil A, Wargnier J, Seriyes H, Berville A (2012) Transfer of architectural traits from perennial *Helianthus mollis* Lam. to sunflower (*H. annuus* L.) and localisation of introgression. Euphytica 186(2):557–572. https://doi.org/10.1007/s10681-012-0656-6

Bryant D, Moulton V (2004) Neighbor-net: an agglomerative method for the construction of phylogenetic networks. Mol Biol Evol 21:255–265. https://doi.org/10.1007/3-540-45784-4_28

Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu Rev Plant Biol 58:377–406. https://doi.org/10.1146/annurev.arplant.58.032806.103835

Chen L, Lou Q, Zhuang Y, Chen J, Zhang X, Wolukau JN (2007) Cytological diploidization and rapid genome changes of the newly synthesized allotetraploids Cucumis x hytivus. Planta 225:603–614. https://doi.org/10.1007/s00425-006-0381-2

Choulet F, Alberti A, Theil S et al (2014) Structural and functional partitioning of bread wheat chromosome 3B. Science 345: 1249721. https://doi.org/10.1126/science.1249721

Comai L (2000) Genetic and epigenetic interactions in allopolyploid plants. Plant Mol Biol 43:387–399. https://doi.org/10.1023/A:1006480722854

Delgado A, Carvalho A, Martín AC, Martín A, Lima-Brito J (2017) Genomic restructuring in $F_1$ Hordeum chilense × durum wheat hybrids and corresponding hexaploid tritordeum lines revealed by DNA fingerprinting analyses. J Genet 96:e13–e24. https://doi.org/10.1007/s12041-017-0772-0

Demirel U, Tındaş I, Yavuz C, Baloch FS (2018) Assessing genetic diversity of potato genotypes using inter-PBS retrotransposon marker system. Plant Genet Res 16:137–145. https://doi.org/10.1017/S1479262117000041

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM (1997) Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics 147:1381–1387

Giordani T, Cavallini A, Natali L (2014) The repetitive component of the sunflower genome. Curr Plant Biol 1:45–54. https://doi.org/10.1016/j.cpb.2014.05.001

Grandbastien MA (1998) Activation of plant retrotransposons under stress conditions. *Trends* Plant Sci 3:181–187. https://doi.org/10.1016/S1360-1385(98)01232-1

Guo DL, Guo MX, Hou XG, Zhang GH (2014) Molecular diversity analysis of grape varieties based on iPBS markers. Bioc Syst Ecol 52:27–32. https://doi.org/10.1016/j.bse.2013.10.008

Hegarty MJ, Barker GL, Wilson ID, Abbott RJ, Edwards KJ, Hiscock SJ (2006) Transcriptome shock after interspecific hybridization in Senecio is ameliorated by genome duplication. Curr Biol 16:1652–1659. https://doi.org/10.1016/j.cub.2006.06.071

Hegarty MJ, Barker GL, Brennan AC, Edwards KJ, Abbott RJ, Hiscock SJ (2008) Changes to gene expression associated with hybrid speciation in plants: further insights from transcriptomic studies in Senecio. Philos Trans R Soc Lond Ser B Biol Sci 363:3055–3069. https://doi.org/10.1098/rstb.2008.0080

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23:254–267. https://doi.org/10.1093/molbev/msj030

Kalendar R, Antonius K, Smýkal P, Schulman AH (2010) iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theor Appl Genet 121:1419–1430. https://doi.org/10.1007/s00122-010-1398-2

Kashkush K, Feldman M, Levy AA (2002) Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. Genetics 160:1651–1659

Kawakami T, Dhakal P, Katterhenry AN, Heatherington CA, Ungerer MC (2011) Transposable element proliferation and genome expansion are rare in contemporary sunflower hybrid populations despite widespread transcriptional activity of LTR retrotransposons. Genome Biol Evol 3:56–167. https://doi.org/10.1093/gbe/evr005

Koh J, Soltis PS, Soltis DE (2010) Homeolog loss and expression changes in natural populations of the recently and repeatedly formed allotetraploid Tragopogon mirus (Asteraceae). BMC Genomics 11:97. https://doi.org/10.1186/1471-2164-11-97

Liu J, Xu X, Xiuxin DX (2005) Intergeneric somatic hybridization and its application to crop genetic improvement. Plant Cell Tissue Organ Cult 82:19–44. https://doi.org/10.1007/s11240-004-6015-0

Liu Z, Seiler GJ, Gulya TJ, Feng J, Rashid KY, Cai X, Jan CC (2017) Triploid production from interspecific crosses of two diploid perennial Helianthus with diploid cultivated sunflower (*Helianthus annuus* L.). G3 (Bethesda) 37(4):1097–1108. https://doi.org/10.1534/g3.116.036327

Ma J, Devos KM, Bennetzen JL (2004) Analyses of LTR retrotransposon structures reveal recent and rapid genomic DNA loss in rice. Genome Res 14:860–869. https://doi.org/10.1101/gr.1466204

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801. https://doi.org/10.1126/science.15739260

Mehmood A, Jaskani MJ, Ahmad S, Ahmad R (2013) Evaluation of genetic diversity in open pollinated guava by iPBS primers. Pak J Agric Sci 50:591–597

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RESEARCH ARTICLE

# On the diversity and origin of the barley complex *agriocrithon* inferred by iPBS transposon markers

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**Abstract** The six-rowed barley with brittle rachis *Hordeum agriocrithon* A.E. Åberg is a diverse taxon connected with the evolution and domestication processes in the genus *Hordeum*. However, the origin and patterns of taxonomic divergence of this barley type is still hotly disputed. Here we utilized inter-primer binding site (iPBS) retrotransposon marker analysis to assess the genetic diversity of the complex taxonomic group *H. agriocrithon* in reference to the other two main barley taxa *H. spontaneum* K. Koch and *H. vulgare* L.. Based on selected iPBS primers, we demonstrated that the long-term process of natural evolution and human-driven domestication have influenced the dynamics of the TE fraction of the barley genome. iPBS markers reliably differentiated at molecular level the three main *Hordeum* taxa but also revealed genetic and phenotypic diversity patterns at within taxon level. Although *H. agriocrithon* can be considered as an autochthonous species due to its genetic divergence from *H. spontaneum* and *H. vulgare*, our results have shown that most of representatives of this taxon are of hybrid origin and hybridization events have shaped its highly heterogeneous genetic structure. Furthermore, our results strongly support the notion that *H. spontaneum* accessions are ancestors of the *H. agriocrithon* subgroup *paradoxon* and that the Caspian sea region is the likely place of initial cultivation and domestication of the six-rowed barley.

**Keywords** Inter-primer binding site · Transposable elements · Genetic diversity · Origin of six-rowed barley · Domestication · Evolution

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## Introduction

In 1930s, a six-rowed barley with a brittle rachis phenotype was first described based on material collected from Tibet (Åberg 1938) and soon after this barley type was assigned the status of a separate species *Hordeum agriocrithon* A.E. Åberg (Åberg 1940). It is characterized by a substantial

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occasional hybridization events between the wild barley and the six-rowed cultivated barley. However, further studies on larger number of accessions are required to shed light on those putative parental forms. We also found *H. agriocrithon* accessions of the *paradoxon* morphotype with distinct genetic structure that appears to have non-hybrid nature. Our results support the view that *H. spontaneum* accessions are ancestors of the proposed *paradoxon* accessions and that the Caspian sea region is the likely place of initial cultivation and domestication of the six-rowed barley.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

Åberg E (1938) *Hordeum agriocrithon*, a wild six-rowed barley. Ann Agr Coll Sweden 6:159–216

Åberg E (1940) The taxonomy and phylogeny of *Hordeum* L. sect. Cerealia Ands. Syrnbolae Bot. Upsalienses, 4(2):1–156

Al-Beyroutiová M, Sabo M, Sleziak P, Dušinský R, Birčák E, Hauptvogel P, Kilian A, Švec M (2016) Evolutionary relationships in the genus Secale revealed by DArTseq DNA polymorphism. Plant Syst Evol 302:1083–1091. https://doi.org/10.1007/s00606-016-1318-2

Andeden EE, Baloch FS, Derya M, Kilian B, Özkan H (2013) iPBS-Retrotransposons-based genetic diversity and relationship among wild annual *Cicer* species. J Plant Biochem Biotechnol 22:453–466. https://doi.org/10.1007/s13562-012-0175-5

Azhaguvel P, Komatsuda T (2007) A phylogenetic analysis based on nucleotide sequence of a marker linked to the brittle rachis lucus indicates a diphyletic origin of barley. Ann Bot 100:1009–1015. https://doi.org/10.1093/aob/mcm129

Baloch FS, Alsaleh A, de Miera LES, Hatipoğlu R, Ciftci V, Karakoy T, Yıldız M, Ozkan H (2015a) DNA based iPBS-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from Turkey. Biochem Syst Ecol 61:244–252. https://doi.org/10.1016/j.bse.2015.06.017

Baloch FS, Derya M, Andeden EE, Alsaleh A, Comertpay G, Kilian B, Ozkan H (2015b) Inter-primer binding site retrotransposon and inter-simple sequence repeat diversity among wild *Lens* species. Biochem Syst Ecol 58:162–168. https://doi.org/10.1016/j.bse.2014.12.002

Baranek M, Meszaros M, Sochorova J, Čechova J, Raddova J (2012) Utility of retrotransposon-derived marker systems for differentiation of presumed clones of the apricot cultivar Velkopavlovicka. Sci Hortic 143:1–6. https://doi.org/10.1016/j.scienta.2012.05.022

Belyayev A (2014) Bursts of transposable elements as an evolutionary driving force. J Evol Biol 27(12):2573–2584. https://doi.org/10.1111/jeb.12513

Bonchev G, Dusinský R, Hauptvogel P, Švec M (2017) Patterns of evolutionary trajectories and domestication history within the genus *Hordeum* assessed by REMAP markers. J Mol Evol 84(2–3):116–128. https://doi.org/10.1007/s00239-016-9779-z

Buckler ES, Thornsberry JM, Kresovich S (2011) Molecular diversity, structure and domestication of grasses. Genet Res 77:213–218. https://doi.org/10.1017/S0016672301005158

Dai F, Nevo E, Wu D, Comadran J, Zhou M, Qiu L, Chen Z, Beiles A, Chen G, Zhang G (2012) Tibet is one of the centers of domestication of cultivated barley. Proc Natl Acad Sci USA 109:16969–16973. https://doi.org/10.1073/pnas.1215265109

Demirel U, Tındaş I, Yavuz C, Baloch FS (2018) Assessing genetic diversity of potato genotypes using inter-PBS retrotransposon marker system. Plant Genet Res 16:137–145. https://doi.org/10.1017/S1479262117000041

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Feng ZY, Liu XJ, Zhang YZ, Ling HQ (2006) Genetic diversity analysis of Tibetan wild barley using SSR markers. Acta Genet Sin 33:917–928. https://doi.org/10.1016/S0379-4172(06)60126-1

Ferreira JR, Pereira JF, Turchetto C, Minella E, Consoli L, Delatorre CA (2016) Assessment of genetic diversity in Brazilian barley using SSR markers. Genet Mol Biol 39(1):86–96. https://doi.org/10.1590/1678-4685-GMB-2015-0148

Grandbastien MA, Audeon C, Bonnivard E et al (2005) Stress activation and genomic impact of Tnt1 retrotransposons in Solanaceae. Cytogenet Genome Res 110:229–241. https://doi.org/10.1159/000084957

Guo DL, Guo MX, Hou XG, Zhang GH (2014) Molecular diversity analysis of grape varieties based on iPBS markers. Bioc Syst Ecol 52:27–32. https://doi.org/10.1016/j.bse.2013.10.008

Hirochika H (1993) Activation of tobacco retrotransposons during tissue culture. EMBO J 12:2521

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23(2):254–267. https://doi.org/10.1093/molbev/msj030

Jilal A, Grando S, Henry RJ, Lee LS, Rice NF, Hill H, Baum M, Ceccarelli S (2008) Genetic diversity of ICARDA's worldwide barley landrace collection. Genet Resour Crop

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ORIGINAL ARTICLE

# Patterns of Evolutionary Trajectories and Domestication History within the Genus *Hordeum* Assessed by REMAP Markers

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**Abstract** The patterns of genetic diversity related to the taxonomy and domestication history of 85 accessions representing the main four species of the genus *Hordeum* were examined by retrotransposon-microsatellite amplified polymorphism (REMAP) markers based on the retrotransposon BARE-1. A substantial level of genetic polymorphisms at among- and within-species level was observed showing that this retrotransposon family and its adjacent genomic regions has been a target for genome dynamics during the evolution and domestication of barley. The obtained data are consistent with the current taxonomic status within the genus *Hordeum*. Similar level of genetic diversity was observed between the wild and the domesticated barley accessions suggesting that transposable elements' activity and accumulation may counteract the decrease of genome-wide diversity following domestication. In addition, eco-geographical sub-genome pools of the cultivated barley were identified in support to the theory of multiple origins of domestication within the genus *Hordeum*. We also provide conclusions about the relationship between accessions of different species and the putative routes of barley domestication. In conclusion, the retrotransposon BARE-1 stands as a reliable and perspective DNA marker for the assessment of the phylogenetic and domestication history in the genus *Hordeum* and other crop species.

**Keywords** Barley · Domestication · BARE-1 · Genetic diversity · Population structure

## Introduction

Domestication is the outcome of selection processes that leads to plants adapted to cultivation and utilization by humans. The understanding of the nature of genetic changes associated with domestication has long been an area of interest and would answer important questions about the geographical origins of species, routes of domestication, and evolution of traits. Plant domestication and diversification lead to profound genetic and phenotypic changes in cultivated forms in comparison to wild ancestors (reviewed in Doebley et al. 2006; Meyer et al. 2012; Meyer and Purugganan 2013; Olsen and Wendel 2013; Pourkheirandish and Komatsuda 2007; Sakuma et al. 2011). The major consequence of the domestication is the reduction of genetic diversity in cultivated species, and this process can take two forms. There is often a genome-wide reduction in neutral diversity by genetic drift-induced bottleneck effects which extent depends on both the number of individuals in the founding population and the duration of the bottleneck. An even greater reduction of diversity, however, can occur in specific genome regions referring to a particular phenotype (or genotype) subjected to selection by human activity. This positive (directional) selection leads to an increase of

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decrease in TE-genetic diversity in the domesticated barley compared to its wild relative seems to be influenced by counteracting effects of domestication-related processes of inter-species hybridization and changes in the environmental conditions. These factors are widely known as a stress factor capable of triggering the activation and accumulation of TEs. Our results also support the hypothesis of the multiple origins of domestication within the genus *Hordeum* and provided further insight into the routes of barley domestication. Therefore, we strongly believe that TE-based markers deserve to find a larger place in the agenda of evolutionary biologists and ecologists.

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**Compliance with Ethical Standards**

**Conflict of interest** The authors declare that there is no conflict of interests.

## References

Abay F, de Boef W, Bjornstad A (2011) Network analysis of barley-seed flows in Tigray, Ethiopia: supporting the design of strategiesthat contribute to on-farm management of plant genetic resources. Plant Gen Res Charact Util 9:495–505.

Azhaguvel P, Komatsuda T (2007) A phylogenetic analysis based on nucleotide sequence of a marker linked to the brittle rachis locus indicates a diphyletic origin of barley. Ann Bot 100:1009–1015

Badr A, Muller K, Schafer Pregl R et al (2000) On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol 17:499–510.

Bellucci E, Bitocchi E, Ferrarini A et al (2014) Decreased nucleotide and expression diversity and modified coexpression patterns characterize domestication in the common bean. Plant Cell 26:1901–1912

Ben Naceur A, Chaabane R, El Falah M et al (2011) Genetic diversity analysis of North Africa's barley using SSR markers. J Genet Eng Biotechnol 10:16–21

Benjak A, Boure S, Forneck A, Casacuberta JM (2009) Recent amplification and impact of MITEs on the genome of grapevine (*Vitis vinifera* L.). Genome Biol Evol 1:75–84

Bennetzen JL, Wang H (2014) The contributions of transposable elements to the structure, function, and evolution of plant genomes. Annu Rev Plant Biol 65:505–530

Bonchev G, Parisod C (2013) Transposable elements and microevolutionary changes in natural populations. Mol. Ecol Res 13:765–775

Bourguiba H, Audergon JM, Krichen L et al (2012) Loss of genetic diversity as a signature of apricot domestication and diffusion into the Mediterranean Basin. BMC Plant Biol 12:49

Buckler ES, Thornsberry JM, Kresovich S (2011) Molecular diversity, structure and domestication of grasses. Genet Res 77:213–218

Caicedo A, Williamson S, Hernandez RD et al (2007) Genome-wide patterns of nucleotide polymorphism in domesticated rice. PLoS Genet 3:e163

Caldwell KS, Russell J, Langridge P, Powell W (2006) Extreme population-dependent linkage disequilibrium detected in an inbreeding plant species, Hordeum vulgare. Genetics 172:557–567

Casacuberta E, Gonzalez J (2013) The impact of transposable elements in environmental adaptation. Mol Ecol 22:1503–1517

Dai F, Nevo E, Wu D et al (2012) Tibet is one of the centers of domestication of cultivated barley. Proc Natl Acad Sci USA 109:16969–16973

Doebley J, Gaut BS, Smith BD (2006) The molecular genetics of crop domestication. Cell 127:1309–1321

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Fang Z, Gonzales AM, Clegg MT et al (2014) Two genomic regions contribute disproportionately to geographic differentiation in wild Barley. G3 4:1193–203.

Fay JC, Wu C-I (2000) Hitchhiking under positive Darwinian selection. Genetics 2000:1405–1413

Feng ZY, Liu XJ, Zhang Y, Ling HQ (2006) Genetic diversity analysis of Tibetan wild barley using SSR markers. Acta Genet Sin 33:917–928

Fischbeck G (2002) Contribution of barley to agriculture: a brief overview. In: Slafer G, Molina-Cano JS, Savin R, Araus JL, Romagosa I (eds) Barley science recent advances from molecular biology to agronomy of yield and quality. Food Products Press, New York, pp 1–29

Freisleben R (1940) Die phylogenetische Bedeutung asiatischer Gersten. Der Zuchter 12:257–272.

Gaut BS, Clegg MT (1993) Nucleotide polymorphism in the Adh1 locus of pearl-millet (*Pennisetum glaucum*) (Poaceae). Genetics 135:1091–1097

Germplasm Resources Information Network [GRIN] (2013). USDA, ARS, National Genetic Resources Program. National Germplasm Resources Laboratory, Beltsville, Maryland. http://www.ars-grin.gov/

Glemin S, Bataillon T (2009) A comparative view of the evolution of grasses under domestication. New Phytol 183:273–290

Gribbon BM, Pearce SR, Kalendar R et al (1999) Phylogeny and transpositional activity of Ty1-copia group retrotransposons in cereal genomes. Mol Gen Genet 261:883–891

Haudry A, Cenci A, Ravel C et al (2007) Grinding up wheat: a massive loss of nucleotide diversity since domestication. Mol Biol Evol 24:1506–1517

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23:254–267

Hyten DL, Song Q, Zhu Y et al (2006) Impacts of genetic bottlenecks on soybean genome diversity. Proc Natl Acad Sci USA 103:16666–16671

Iqbal MJ, Reddy O, El-Zik KM, Pepper AE (2001) A genetic bottleneck in the 'evolution under domestication' of upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. Theor Appl Genet 103:547–554

Jones H, Civán P, Cockram J et al (2011) Evolutionary history of barley cultivation in Europe revealed by genetic analysis of extant landraces. BMC Evol Biol 11:320

Jones G, Jones H, Charles MP et al (2012) Phylogeographic analysis of barley DNA as evidence for the spread of Neolithic agriculture through Europe. J Archaeol Sci 39:3230–3238

Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A (1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor App Genet 98:704–711.

Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH (2000) Genome evolution of wild barley (*Hordeum spontaneum*) by *BARE*-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc Natl Acad Sci USA 97:6603–6607

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PLANT GENETICS • ORIGINAL PAPER

# Genomic diversity of *Ac*-like transposable elements in *sphaerococcum* mutant forms of common wheat (*Triticum aestivum* L.) and triticale (X *Triticosecale* Witt.)

**Georgi Bonchev · Lubomir Stoilov · Zorniza Angelova · Sevdalin Georgiev**

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**Abstract** DNA sequences homologous to the maize Activator (*Ac*) element are widespread in plant genomes. Nowadays, several reports are available concerning the distribution and characterisation of *Ac*-homologous sequences in natural populations of different cereal species. but these mobile genetic elements still remain to be comprehensively characterised. In this respect, there is a particular lack of information about the dynamics of *Ac*-homologous sequences within mutant germplasm collections. Here, we present data on the genomic diversity and methylation patterns of *Ac*-homologous sequences in ethyl methanesulphonate (EMS)-induced *sphaerococcum* mutant forms of common wheat (*Triticum aestivum* L.) and triticale (X *Triticosecale* Witt.). The results show that the initial EMS treatment has influenced the wheat genome stability by enhancing the dynamics of *Ac* transposon-homologous sequences.

**Keywords** Transposons · Wheat · Triticale · *Sphaerococcum* mutants · Genomic instability

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## Introduction

In many plant species with large and complex genomes, like that of wheat, the mobile genetic elements or transposons comprise more than 50% of the nuclear DNA (Sanmiguel and Bennetzen 1998; Li et al. 2004). Their activity is often associated with changes in the gene or genome structure, accompanied with the modulation of gene expression in both germinal and somatic plant cells (Bennetzen 2000). For this reason, Barbara McClintock named the transposons 'controlling elements' and proposed their role in evolution as a ubiquitous source of hypermutagenicity and for the generation of individuals with increased survival potential within stressed populations (McClintock 1949).

The *hobo/Ac/Tam3* (hAT) superfamily of transposons is a major group of class II elements (Calvi et al. 1991), which is known to be responsible for diverse morphological and chromosomal mutations. Members of the hAT family (*Ac*-like transposable elements) are widespread in plants, particularly in large cereal genomes (Kunze et al. 1997; Staginnus et al. 2001; Langdon et al. 2003), and can be recognised by structural similarity in their terminal inverted repeats (TIR) or internal transposase coding region. The sequence coding for the maize transposase is the most conservative region of *Ac*-like transposable elements (Rubin et al. 2001). Based on this feature, several approaches (e.g. database homology searches, Southern hybridisation and polymerase chain reaction [PCR] amplification) have been applied for their detection in cereal genomes (Chernyshev et al. 1989; Georgiev et al. 2000; Zale and Steber 2002; De Keukeleire et al. 2004; Altinkut et al. 2006a, 2006b). Beside these reports, there are no data available regarding the structural and functional dynamics of these elements in mutant wheat genomes, as the majority

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## References

Altinkut A, Kotseruba V, Kirzhner VM, Nevo E, Raskina O, Belyayev A (2006a) *Ac*-like transposons in populations of wild diploid Triticeae species: comparative analysis of chromosomal distribution. Chromosome Res 14:307–317. doi:10.1007/s10577-006-1048-3

Altinkut A, Raskina O, Nevo E, Belyayev A (2006b) *En/Spm*-like transposons in Poaceae species: transposase sequence variability and chromosomal distribution. Cell Mol Biol Lett 11:214–230. doi:10.2478/s11658-006-0017-3

Bennetzen JL (2000) Transposable element contributions to plant gene and genome evolution. Plant Mol Biol 42:251–269

Bonchev G, Georgiev S, Pearce S (2010) Retrotransposons and ethyl methanesulfonate-induced diversity in hexaploid wheat and *Triticale*. Cent Eur J Biol 5:765–776. doi:10.2478/s11535-010-0072-7

Calvi BR, Hong TJ, Findley SD, Gelbart WM (1991) Evidence for a common evolutionary origin of inverted repeat transposons in Drosophila and plants: hobo, Activator, and Tam3. Cell 66:465–471. doi:10.1016/0092-8674(81)90010-6

Chernyshev AI, Golovkin MV, Mil'shina NV, Gazumyan AK, Anan'ev EV (1989) Molecular-genetic organization of mobile elements of the *Ac-Ds* family in cereal genomes. Identification of DNA sequences homologous to the *Ac* element of maize in barley *Hordeum vulgare* L. genome. Sov Genet 24:1338–1344

De Keukeleire P, De Schepper S, Gielis J, Gerats T (2004) A PCR-based assay to detect *hAT*-like transposon sequences in plants. Chromosome Res 12:117–123. doi:10.1023/B:CHRO.0000013163.34505.96

Dvorak J, Akhunov ED, Akhunov AR, Deal KR, Luo MC (2006) Molecular characterization of a diagnostic DNA marker for domesticated tetraploid wheat provides evidence for gene flow from wild tetraploid wheat to hexaploid wheat. Mol Biol Evol 23:1386–1396. doi:10.1093/molbev/msl004

Fedoroff N, Wessler S, Shure M (1983) Isolation of the transposable maize controlling elements *Ac* and *Ds*. Cell 35:235–242. doi:10.1016/0092-8674(83)90226-X

Finnegan EJ, Genger RK, Peacock WJ, Dennis ES (1988) DNA methylation in plants. Annu Rev Plant Physiol Plant Mol Biol 49:223–247. doi:10.1146/annurev.arplant.49.1.223

Georgiev S (1976) EMS-induced mutants of the *sphaerococcum* type in *T. aestivum* L. Genet Plant Breed 9:218–227

Georgiev S (1982) EMS-induced *sphaerococcum* mutation in Triticale. Wheat Inf Serv, Japan 55:32–35

Georgiev S, Nicoloff H (1976) Cytological study of certain *sphaerococcum* type of mutant forms obtained from *Triticum aestivum* L. Proc Bulg Acad Sci 29:1681–1684

Georgiev S, Dekova T, Atanassov I, Angelova Z, Dimitrova A, Mirkova V, Stoilov L (2000) Transposable elements in wheat and *Triticale sphaerococcum* mutant forms. Biotechnol Biotechnol Equip 14:25–32

Georgiev S (2004) Molecular genetic and cytogenetic studies of functional activity of the genomes of some representatives of *Triticum*, *Hordeum* and *Silene* L. D. Sc Dissertation, Sofia University "St. Kliment Ochridski"

Kunze R, Saedler H, Lönnig W-E (1997) Plant transposable elements. Adv Bot Res 27:331–470. doi:10.1016/S0065-2296(08)60284-0

Langdon T, Jenkins G, Hasterok R, Jones RN, King IP (2003) A high-copy-number CACTA family transposon in temperate grasses and cereals. Genetics 163:1097–1108

Li W, Zhang P, Fellers JP, Friebe B, Gill BS (2004) Sequence composition, organization, and evolution of the core Triticeae genome. Plant J 40:500–511. doi:10.1111/j.1365-313X.2004.02228.x

McClintock B (1949) Mutable loci in maize. Carnegie Inst Washington Year Book 48:142–154

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801. doi:10.1126/science.15739260

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 19:4321–4325. doi:10.1093/nar/8.19.4321

Rubin E, Lithwick G, Levy AA (2001) Structure and evolution of the hAT transposon superfamily. Genetics 158:949–957

Sanmiguel P, Bennetzen JL (1998) Evidence that a recent increase in maize genome size was caused by the massive amplification of intergene retrotransposons. Ann Bot 82:37–44

Sears ER (1947) The *sphaerococcum* gene in wheat. Genetics 32:102–103

Slotkin RK, Martienssen R (2007) Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet 8:272–285. doi:10.1038/nrg2072

Staginnus CB, Huettel CD, Desel C, Schmidt T, Kahl G (2001) A PCR-based assay to detect *En/Spm*-like transposon sequences in plants. Chromosome Res 9:591–605. doi:10.1023/A:1012455520353

Wang L, Heinlein M, Kunze R (1996) Methylation pattern of Activator transposase binding sites in maize endosperm. Plant Cell 8:747–758. doi:10.1105/tpc.8.4.747

Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25:1189–1191. doi:10.1093/bioinformatics/btp033

Zale JM, Steber CM (2002) Transposon-related sequences in the Triticeae. Cereal Res Commun 30:237–244

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# Morphological, Pathological and Genetic Diversity of the *Colletotrichum* Species, Pathogenic on Solanaceous Vegetable Crops in Bulgaria

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**Abstract:** *Colletotrichum* species are among the most devastating plant pathogens in a wide range of hosts. Their accurate identification requires a polyphasic approach, including geographical, ecological, morphological, and genetic data. Solanaceous crops are of significant economic importance for Bulgarian agriculture. *Colletotrichum*-associated diseases pose a serious threat to the yield and quality of production but are still largely unexplored. The aim of this study was to identify and characterize 26 pathogenic *Colletotrichum* isolates that threaten solanaceous crops based on morphological, pathogenic, and molecular data. DNA barcodes enabled the discrimination of three main taxonomic groups: *C. acutatum*, *C. gloeosporioides*, and *C. coccodes*. Three different species of acutatum complex (*C. nymphaeae*, *C. godetiae*, and *C. salicis*) and *C. cigarro* of the gloeosporioides complex were associated with fruit anthracnose in peppers and tomatoes. The *C. coccodes* group was divided in two clades: *C. nigrum*, isolated predominantly from fruits, and *C. coccodes*, isolated mainly from roots. Only *C. salicis* and *C. cigarro* produced sexual morphs. The species *C. godetiae*, *C. salicis*, and *C. cigarro* have not previously been reported in Bulgaria. Our results enrich the knowledge of the biodiversity and specific features of *Colletotrichum* species, which are pathogenic to solanaceous hosts, and may serve as a scientific platform for efficient disease control and resistance breeding.

**Keywords:** phytopathogenic fungi; *Colletotrichum nymphaeae*; *Colletotrichum godetiae*; *Colletotrichum salicis*; *Colletotrichum cigarro*; multilocus DNA barcoding; genotyping



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## 1. Introduction

Species belonging to the genus *Colletotrichum* are among the most important plant pathogens worldwide from both a scientific and an economic point of view. They affect a wide range of host plants, causing various diseases to all plant organs, with anthracnose as the main devastating disease damaging the aboveground parts of plants, such as fruits, leaves, flowers, and stems [1]. Symptoms of anthracnose fruit rot frequently develop during the postharvest period, not only causing enormous losses in production quality but also possibly having direct implications for human health [2]. Although plants are the main hosts of *Colletotrichum* species, recent studies have shown that insects, animals, and even humans might be infected [3,4]. Therefore, the fight against *Colletotrichum* phytopathogens is of great importance for sustainable crop production and global food safety [5]. Effective tools for accurate species identification and characterization are required to obtain insight into the biology of the genus, the specificity of pathogen–host interactions, and detailed

caused the development of large necrotic spots, but with a different morphology. The lowest virulence of the *C. coccodes* isolates could be explained by a greater host specificity to some members of the Solanaceae family.

## 5. Conclusions

In conclusion, our study confirmed the applicability of morphological, cultural, and DNA sequence data to clarify relationships among the isolates studied. The obtained results add to previous reports about the relevance of DNA barcodes as useful markers for describing genetic diversity and species delimitation within the genus *Colletotrichum*. Our findings showed that three different species of the acutatum complex (*C. nymphaeae*, *C. godetiae*, and *C. salicis*) were associated with anthracnose fruit rot in peppers and tomatoes. The gloeosporioides complex was represented by four phenotypically and genotypically homogeneous isolates, defined as *C. cigarro*, and also related to fruit anthracnose of pepper and tomato plants. Applying ACT and TUB2 as secondary barcodes, the *coccodes* group was divided into two clades: *C. nigrum* and *C. coccodes*, showing good agreement with previous findings by other authors. *C. nigrum* was isolated predominantly from the fruits of the studied hosts, and *C. coccodes* was mainly isolated from the roots. In this study, only two *Colletotrichum* species (*C. salicis* and *C. cigarro*) produced sexual morphs in culture. The advantages demonstrated by the species *C. cigarro*, which has the ability to easily form a sexual morph and to attack a wide range of hosts, make it a threat to other hosts beyond the Solanaceae family. The species *C. godetiae*, *C. salicis*, and *C. cigarro* have not previously been reported as naturally occurring plant pathogens in Bulgaria. The identification of these species allows us to update the list of *Colletotrichum* species found in the region, and shows the extended geographical range of their distribution worldwide. Our results enrich the knowledge about the biodiversity and the specific features of the *Colletotrichum* species, which are pathogenic for the solanaceous hosts in Bulgaria, and serve as a scientific basis for efficient disease control and resistance breeding.

**Author Contributions:** Conceptualization, G.B., V.M., Z.S. and R.R.; methodology, V.M., R.R., Z.S. and G.B.; investigation, V.M., Z.S., R.R. and I.B.; writing—original draft preparation, V.M., R.R., Z.S. and G.B.; writing—review and editing, V.M., R.R., Z.S., G.B., I.B., H.K., E.V. and H.W.; project administration, G.B.; funding acquisition, G.B. and R.R. All authors have read and agreed to the published version of the manuscript.

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## Appendix A

The consensus tree (Figure A1) was drawn using Geneious tree builder (Tamura-Nei genetic distance model, UPGMA tree building method, 1000 Bootstrap replicates, consensus method: majority greedy clustering) [82].

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# Catmint (*Nepeta nuda* L.) Phylogenetics and Metabolic Responses in Variable Growth Conditions

***Detelina Petrova*** [1], ***Uroš Gašić*** [2], ***Lyubomira Yocheva*** [3], ***Anton Hinkov*** [4], ***Zhenya Yordanova*** [1], ***Ganka Chaneva*** [1], ***Desislava Mantovska*** [1], ***Momchil Paunov*** [5], ***Lyubomira Ivanova*** [1], ***Mariya Rogova*** [1], ***Kalina Shishkova*** [4], ***Daniel Todorov*** [4], ***Anita Tosheva*** [6], ***Veneta Kapchina-Toteva*** [1], ***Valya Vassileva*** [7], ***Atanas Atanassov*** [8], ***Danijela Mišić*** [2], ***Georgi Bonchev*** [7]* ***and Miroslava Zhiponova*** [1]*

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*Nepeta nuda* (catmint; Lamiaceae) is a perennial medicinal plant with a wide geographic distribution in Europe and Asia. This study first characterized the taxonomic position of *N. nuda* using DNA barcoding technology. Since medicinal plants are rich in secondary metabolites contributing to their adaptive immune response, we explored the *N. nuda* metabolic adjustment operating under variable environments. Through comparative analysis of wild-grown and *in vitro* cultivated plants, we assessed the change in phenolic and iridoid compounds, and the associated immune activities. The wild-grown plants from different Bulgarian locations contained variable amounts of phenolic compounds manifested by a general increase in flowers, as compared to leaves, while a strong reduction was observed in the *in vitro* plants. A similar trend was noted for the antioxidant and anti-herpesvirus activity of the extracts. The antimicrobial potential, however, was very similar, regardless the growth conditions. Analysis of the *N. nuda* extracts led to identification of 63 compounds including phenolic acids and derivatives, flavonoids, and iridoids. Quantification of the content of 21 target compounds indicated their general reduction in the extracts from *in vitro* plants, and only the ferulic acid (FA) was specifically increased. Cultivation of *in vitro* plants under different light quality and intensity indicated that these variable light conditions altered the content of bioactive compounds, such as aesculin, FA, rosmarinic acid, cirsimaritin, naringenin, rutin, isoquercetin, epideoxyloganic acid, chlorogenic acid. Thus, this study generated novel information on the regulation of *N. nuda* productivity using light and other cultivation conditions, which could be exploited for biotechnological purposes.

**Keywords: antibacterial, antioxidant, antiviral, DNA barcoding, iridoids, light, *Nepeta nuda*, phenolics**

**TABLE 5** | Continued

| Biological activities | Phenolics and iridoids in *N. nuda* | Extract details | References |
|---|---|---|---|
| *S. aureus*, *B. cereus* | Nepetoidin B | *Isodon lophathoides* var. *graciliflorus* (Bentham) H. Hara (Lamiaceae) | Zhou et al., 2014 |
| oral pathogens | RA, verminoside | *N. nuda* tincture | Smiljković et al., 2018 |
| *S. aureus* | Phenols and flavonoids | *N. nuda* ethanol extract | Dordević et al., 2019 |
| B. cereus, S. aureus | Nepetalactones, 1,5,9-ELA, RA | *N. rtanjensis* Diklić and Milojević and *N. argolica* Bory and Chaub. in Bory subsp. *argolica* methanol extracts and pure iridoids | Aničić et al., 2021 |
| *A. calcoaceticus*, *K. pneumoniae*, *B. cereus*, *S. aureus* | FA, gallic acid | *N. nuda* subsp. *nuda* methanol extracts (pretreated with chloroform) | This study |

*na, not applicable.*

## Light-Dependent Metabolic Modulation of *N. nuda* During *in vitro* Cultivation

The light composition and intensity are important factors in stimulating plant flowering (Zhiponova et al., 2020a). The observed metabolite enrichment in *N. nuda* flowers led to the use of the previously reported light formula for boosting of flowering (Zhiponova et al., 2020a) in our *in vitro* experiment. Consistently with the natural environmental adaptation of *N. nuda*, the high light intensity stimulated RA (18), cirsimaritin (50), naringenin (69*), rutin (71*), isoquercetin (72*), as well as 1,5,9-*e*DLA (59). Lower light intensity significantly upregulated the chlorogenic acid (65*), which supports the existence of a positive correlation between the increased production of this metabolite and the low intensity and combination of blue, red and far–red lights (Chen et al., 2016). Aesculin (11) and FA (16) were specific for the W control variant, which could reflect lack of importance for flowering. Indeed, respective decrease (for aesculin) and slightly higher levels (for FA) of these compounds were detected in the flowers, as compared to the leaves.

## Conclusions and Future Perspectives

This study characterized the impact of environmental factors in modulating the content of bioactive compounds in *N. nuda* that is largely distributed in Bulgaria. DNA barcoding enabled us to genetically discriminate and determine the precise phylogenetic position of *N. nuda* using the available *Nepeta* sequence records in public databases. We provided the first DNA barcode records for *N. nuda* in the BOLD database, thus contributing to the enrichment of global catalogs and specific genetic diversity for the genus *Nepeta* in Bulgaria. These data can be a valuable basis for further species identification and support future studies regarding *Nepeta* genetic variability. Organ specificity of phytoimmunity features of *N. nuda*, such as the antioxidant and antiviral activities, phenolic acids, flavonoids and iridoid glycosides, were analyzed and discussed from an ecophysiological perspective, and their dependence on environmental conditions. Furthermore, this study showed that the light spectrum and intensity are crucial factors affecting the differential accumulation of phenolic acids, flavonoids and iridoids in *N. nuda*. Therefore, these studies could strengthen and facilitate the understanding of *N. nuda* ecology and targeted modulation of its productivity under controlled conditions, thus entailing their potential benefits for agriculture and pharmacology.

The current work provides the platform for further detailed studies on *Nepeta* species in multiple research directions. In point of view of genetic diversity, the study of ecological and taxonomic dynamics within the genus *Nepeta* is of particular interest especially when correlating a genetic ecotype and metabolite activities. It would be of interest to define more aspects of the molecular mechanism regulating phenolics and iridoids levels—effect of environmental signals, gene expression, parallel profiles of other *N. nuda* metabolites. Regarding the biological activities, it would be of interest to identify specific related metabolites with importance for food quality and human health. The application of biotechnological approaches would assist targeted accumulation of compounds by elicitors, as well as enhanced metabolic production by cell cultures and bioreactor.

## DATA AVAILABILITY STATEMENT

The DNA barcoding datasets presented in this study can be found in online repository. The names of the repository and accession numbers can be found in the article and **Supplementary Material**, respectively.

## AUTHOR CONTRIBUTIONS

MZ collected the plant material. AT did taxonomic annotation of the plant material and collected botanical information. GB and VV executed DNA barcoding assay. GB and MZ performed the phylogenetic study. ZY and MZ maintained *N. nuda in vitro* cultures. DMa and MR prepared the extracts. DMa and LI did antioxidant analyses. AH, KA, and DT designed and performed antiviral assays. DP and LY designed and tested antibacterial activities. UG and DMi designed and performed UHPLC-LTQ OrbiTrap XL and UHPLC/qqqMS2 assays. GC and MZ performed the light experiments. MP performed the PCA analysis. MZ, DP, AH, VV, AA, DMi, and GB wrote the manuscript. All authors discussed the results and approved the final manuscript.

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ORIGINAL RESEARCH
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# Identification, High-Density Mapping, and Characterization of New Major Powdery Mildew Resistance Loci From the Emmer Wheat Landrace GZ1

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Powdery mildew is one of the most devastating diseases of wheat which significantly decreases yield and quality. Identification of new sources of resistance and their implementation in breeding programs is the most effective way of disease control. Two major powdery mildew resistance loci conferring resistance to all races in seedling and adult plant stages were identified in the emmer wheat landrace GZ1. Their positions, effects, and transferability were verified using two linkage maps (1,510 codominant SNP markers) constructed from two mapping populations (276 lines in total) based on the resistant GZ1 line. The dominant resistance locus *QPm.GZ1-7A* was located in a 90 cM interval of chromosome 7AL and explains up to 20% of the trait variation. The recessive locus *QPm.GZ1-2A*, which provides total resistance, explains up to 40% of the trait variation and was located in the distal part of chromosome 2AL. The locus was saturated with 14 PCR-based markers and delimited to a 0.99 cM region which corresponds to 4.3 Mb of the cv. Zavitan reference genome and comprises 55 predicted genes with no apparent candidate for the *QPm.GZ1-2A* resistance gene. No recessive resistance gene or allele was located at the locus before, suggesting the presence of a new powdery mildew resistance gene in the GZ1. The mapping data and markers could be used for the implementation of the locus in breeding. Moreover, they are an ideal base for cloning and study of host–pathogen interaction pathways determined by the resistance genes.

**Keywords: wheat, powdery mildew (*Blumeria graminis* D. C. f. sp. *tritici*), resistance, emmer, GZ1, QTL mapping**

## INTRODUCTION

Hexaploid bread wheat (*Triticum aestivum* subsp. *aestivum*, 2n = 6× = 42, AABBDD) and tetraploid durum (pasta) wheat (*Triticum turgidum* subsp. *durum*, 2n = 4× = 28, AABB) are significant commercial grain crops worldwide. Their high and stable yield is the most important aspect of food security. Nevertheless, wheat yields could be threatened by various fungal diseases such as powdery mildew, rust, or Fusarium head blight (Chrpová et al., 2013; Bansal et al., 2020;

*QPm.GZ1-7A* is dominant, suggesting that it could be an NBS-LRR-like gene.

The recessive character of *QPm.GZ1-2A* and its strong resistance effect make it a more attractive source of resistance compared to the *QPm.GZ1-7A*, and therefore, only the *QPm.GZ1-2A* was selected for further map saturation. The flanking markers of the GZ1 × EBL region (**Supplementary Tables 1, 3**) were used as the starting points. The 22.7 Mb long *QPm.GZ1-2A* region of the GZ1 × EBL map was saturated with 14 new markers (**Table 1**). The final *QPm.GZ1-2A* region (about 4.3 Mb) in the cv. Zavitan reference genome sequence (Avni et al., 2017) is flanked by the *owm2016* and *1101086-26* markers (**Supplementary Table 7**). The narrowed down *QPm.GZ1-2A* region does not overlap with the QTL peaks (associated with the 41420734-*12* marker, **Figure 2**) predicted by the QTL analysis. The observed proximal shift (**Supplementary Table 7**) could be attributed to noise in the phenotype data caused by the presence of two resistance genes.

The *QPm.GZ1-2A* region comprises 55 annotated genes (**Supplementary Table 3**) and none of them have any relation to the *Mlo* gene family. Additionally, the *Mlo* gene was mapped on chromosome 4H (Simons et al., 1997) and is not orthologous to the *QPm.GZ1-2A* mapped on chromosome 2A supporting the previous assumptions that *QPm.GZ1-2A* is different from the *Mlo* gene. However, three of these genes have relation to the resistance genes involved in pathogen attack signaling. Since genes from the signaling pathways are mostly dominant R-genes (e.g., Peart et al., 2005; Sánchez-Martín et al., 2016), there is only a small probability that the recessive *QPm.GZ1-2A* is one of them. However, one of them could be the *PmHNK54* gene (Xu et al., 2011). Nevertheless, confirmation of the assumptions would require further work.

The broad-range resistance in all stages of plant development mediated by the single major *QPm.GZ1-2A* locus makes it extremely attractive to breeders and the high-density genetic map of the locus offers molecular markers for its effective implementation in breeding programs. Moreover, the results provide an ideal base for cloning and study of the novel recessive gene determining the resistance.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## AUTHOR CONTRIBUTIONS

MV, ZK, and MŠ designed the study. ZK, AL, and MM were responsible for marker development, genotyping, map construction, and data analysis. MŠ and EJ were responsible for the construction of mapping populations. MŠ, GB, and ZK performed the phenotypic evaluation. AL performed the statistical analysis. PC sorted the chromosomes and extracted chromosome-specific DNA. KH was responsible for survey sequencing and sequence assembly. ZK, MV, and JJ conducted the bioinformatics analyses. ZK conducted the other experiments. ZK and MV drafted the manuscript. All authors contributed to its editing and proofreading.

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## SUPPLEMENTARY MATERIAL

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## REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mole. Biol.* 215, 403–410. doi: 10.1016/s0022-2836(05)80360-2

Avni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S. O., Gundlach, H., et al. (2017). Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. *Science* 357, 93–97. doi: 10.1126/science.aan0032

Bansal, M., Adamski, N. M., Toor, P. I., Kaur, S., Molnár, I., Holušová, K., et al. (2020). *Aegilops umbellulata* introgression carrying leaf rust and stripe rust resistance genes *Lr76* and *Yr70* located to 9.47-Mb region on 5DS telomeric end through a combination of chromosome sorting and sequencing. *Theoret. Appl. Genet.* 133, 903–915. doi: 10.1007/s00122-019-03514-x

Bayles, C. J., Ghemawat, M. S., and Aist, J. R. (1990). Inhibition by 2-deoxy-D-glucose of callose formation, papilla deposition, and resistance to powdery mildew in an *ml-o* barley mutant. *Physiol. Mole. Plant Pathol.* 36, 63–72. doi: 10.1016/0885-5765(90)90092-c

Ben-David, R., Xie, W., Peleg, Z., Saranga, Y., Dinoor, A., and Fahima, T. (2010). Identification and mapping of *PmG16*, a powdery mildew resistance gene

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# DNA Barcoding Study of Representative *Thymus* Species in Bulgaria

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**Abstract:** We present a study on the taxonomy of eleven *Thymus* species, belonging to two sections and occurring naturally in Bulgaria. Four DNA barcoding markers—matK, rbcL, trnH-psbA and ITS—were applied to discriminate the species and to reveal their phylogenetic relationships. The results showed that rbcL has the lowest discriminating power regarding the studied species, while the other markers yielded results fitting better to the existing taxonomic schemes based on morphological traits. However, even in the case of better performing markers, the results were not straightforward—morphologically distinct species belonging to different sections were grouped together, and closely related species appeared genetically distinct. The results are typical for taxonomically complex groups, such as the genus *Thymus*, characterized in Bulgaria with great diversity, high percentage of endemism and still requiring a full and comprehensive taxonomic study. The results are discussed in the light of unresolved taxonomic problems and application of DNA barcoding methods.

**Keywords:** genetic markers; taxonomy; medicinal plants; phylogeny; taxonomically complex groups (TCGs)



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## 1. Introduction

Resolving the problems arising when studying taxonomically complex groups (TCGs) requires a combined approach consisting of classical (morphological, anatomical, cytological) and modern (molecular) methods. Representatives of the genus *Thymus* can be a good example of a complex group encompassing many taxa, some of them with uncertain status, related among each other by hybridization, overlapping phenotypic variation and other attributes of the reticulate evolution, making the task of taxonomists more difficult [1].

The complex systematics of the genus *Thymus* has been outlined in many studies attempting to resolve the puzzle or a part of it [2–6]. Most of the challenges still stand today, and in many cases, the application of modern molecular methods did not provide a clear solution to taxonomic problems [1,7].

Currently, the number of species of the genus *Thymus* in Bulgaria is 21 [8–11], and the species list slightly differs from the one in the Euro+Med PlantBase (https://ww2.bgbm.org/EuroPlusMed/; last accessed 23 December 2021). In terms of species diversity, Bulgaria is among the richest countries in Europe (see also [12], for review). The genus *Thymus* is subdivided in two subgenera: *Coridothymus* (Reichenb. f.) Borbás and *Thymus* [2]. All Bulgarian species belong to the nominate subgenus.

Due to the importance of *Thymus* species as medicinal and aromatic plants and because of the conservation value of many species, they always provoked a substantial interest and have been subjected to diverse studies.

**Table 4.** Oligonucleotide primers used and PCR conditions.

| Barcode Region | Primers | Primer Sequences 5′-3′ | PCR Conditions |
|---|---|---|---|
| matK | MatK-RKIM-f | ACCCAGTCCATCTGGAAATCTTGGTTC | 95 °C 5 min<br>95 °C 30 s,<br>51 °C 50 s<br>72 °C 1.4 min, 35 cycles<br>72 °C 7 min |
| | MatK-3FKIM-r | CGTACAGTACTTTTGTGTTTACGAG | |
| rbcL | rbcLa-F | ATGTCACCACAAACAGAGACTAAAGC | 94 °C 4 min<br>94 °C 30 s<br>55 °C 30 s<br>72 °C 1 min, 35 cycles<br>72 °C 10 min |
| | rbcLajf634R | GAAACGGTCTCTCCAACGCAT | |
| trnH-psbA | psbA-trnH | CGCGCATGGTGGATTCACAATCC | 94 °C 4 min<br>94 °C 30 s,<br>55 °C 30 s,<br>72 °C 1 min, 35 cycles<br>72 °C 7 min |
| | psbA-3F | GTTATGCATGAACGTAATGCTC | |
| ITS | ITS_F1 | CCTTATCATTTAGAGGAAGGAG | 94 °C 5 min<br>94 °C 30 s,<br>50 °C 30 s,<br>72 °C 1min, 35 cycles<br>72 °C 5 min |
| | ITS 4 | TCCTCCGCTTATTGATATGC | |

## 4. Conclusions

Low bootstrap support testifies to the unreliability of the majority of groups identified on phylogenetic trees and casts doubt on the possibility of using the studied markers to study phylogenetic relationships in a taxonomically complex group, such as the genus *Thymus*. A weak trend in pooling samples of the same species indicates the low value of the studied markers for barcoding and suggests the need to look for other markers. Future approaches to the study of *Thymus* taxonomy and phylogeny should be more complex, including a set of molecular, morphological and other phenotypic markers. Additionally, population genetic studies could provide a reliable picture of the distribution of genetic diversity and the degree of differentiation and could help delineate the real distinct populations and, possibly, the natural entities at the species level, as suggested by [1].

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11030270/s1.

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RESEARCH ARTICLE

# Neonicotinoid insecticides exert diverse cytotoxic and genotoxic effects on cultivated sunflower

Mariyana Georgieva [1] · Georgi Bonchev [1] · Grigor Zehirov [1] · Vesela Vasileva [1] · Valya Vassileva [2]

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**Abstract**
Contamination with neonicotinoids is a global problem affecting environment and target and non-target organisms including plants. The present study explored the potential genotoxic and cytotoxic effects of the insecticides Actara 25 WD and Nuprid 200 SL containing the active substances thiamethoxam (TMX) and imidacloprid (IMI), respectively, on cultivated sunflower (*Helianthus annuus* L.). The half maximal effective concentration ($½EC_{50}$) of the tested substances was calculated using a dose-response inhibition analysis of the growth of plant roots relative to the corresponding controls. Application of approximately $½EC_{50}$ or higher TMX doses significantly increased the antioxidant activity in sunflower leaves, whereas IMI led to a significant decrease in root antioxidant capacity, indicating organ-specific insecticide effects on sunflower plants. Even low doses ($½EC_{50}$) of the studied neonicotinoids led to irregularities in mitotic phases and abnormalities in the cytokinesis and chromosome segregation, such as bridges, laggards, stickiness, and C-mitosis. Genotoxic effects manifested by a dose-independent induction of primary DNA damages and retrotransposon dynamics were also observed. The used set of physiological, biochemical, and genetic traits provides new information about the organ-specific effects of neonicotinoids in sunflower plants and elaborates on the complexity of mechanisms underpinning these effects that include DNA damages, cytokinesis defects, and genome instability.

**Keywords** Cytotoxicity · DNA damage · Genotoxicity · Lipid peroxidation · Neonicotinoid insecticides · Retrotransposon dynamics

## Introduction

Neonicotinoids are widely used insecticides that have beneficial economic effects based on their effective pest control and contribution to plant vigor and crop yield. However, the extensive use of insecticides in modern agriculture entails risks of their accumulation in soils, groundwater, and plant tissues. Most studies on the biological effects of neonicotinoid insecticides have been conducted with animals, such as soil and aquatic organisms, worms, fish, rodents, and mammals (Dittbrenner et al. 2011; Feng et al. 2005; Finnegan et al. 2017). Uncontrolled dispersion of neonicotinoid insecticides from target fields to surrounding agricultural and urban areas affects various non-target organisms (Malev et al. 2012). The application of these substances on agricultural crops can be hazardous to pollinators, including honeybees (Craddock et al. 2019). The influence of neonicotinoids on different plant traits, however, remains poorly studied and disputable. Despite the low uptake (approximately 5%) of neonicotinoids by crops (Wood and Goulson 2017), they are transported to all plant tissues and intensively metabolized often forming toxic compounds (Simon-Delso et al. 2015). Residue concentrations in plants may range from parts per billion (ppb) to parts per million (ppm) (Bonmatin et al. 2015; Craddock et al. 2019).

**Highlights**
- Neonicotinoid insecticides inhibit root growth in cultivated sunflower.
- Neonicotinoids do not damage cell membranes.
- Neonicotinoids provoke organ-specific antioxidant responses.
- Neonicotinoids exert dose-independent DNA damages and higher transposon dynamics
- Neonicotinoid insecticides affect chromosome segregation and cytokinesis.

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et al. 2014). Laggards and stickiness were very frequent chromosomal aberrations upon exposure to TMX and IMI for 24 h. These defects might be due to inhibition of tubulin proteins, which could disturb spindle formation and interrupt normal chromosome migration to the poles (Kuchy et al. 2016). Indeed, there are two main reasons for the occurrence of C-mitosis in the cells: disturbances of microtubules and distortion of spindle formation (Odeigah et al. 1997). In this study, the chromosome stickiness could be a sign of insecticide toxicity with consequent lethal outcomes for the organisms. The potential causative for this type of aberrations is the emergence of inter- and intra-chromatid crosslinks (Kovalchuk et al. 1998). Although the biochemical nature of this abnormality is still unknown, it has been suggested that chromosome stickiness originates from the functional failure of specific non-histone proteins involved in the segregation and separation of the chromatids (Gaulden 1987). Recent studies on humans have linked the chromosome stickiness with the functional defect in the Ki-67 protein, which prevents the binding of chromosomes after nuclear envelope disintegration (Brangwynne and Marko 2016; Cuylen et al. 2016). Plant analogs of these proteins and genes have not been identified yet. The studied neonicotinoids also induced clastogenic aberrations, such as chromosomal breaks, chromosomal bridges, and ring chromosomes. The formation of chromosome bridges is normally linked to the occurrence of double-stranded DNA breaks. At the end of the cell division, their formation can lead to chromosome or chromatid fragmentation, which in turn are visualized as micronuclei in daughter cells (Luzhna et al. 2013).

In conclusion, this work explored the relatively unknown adverse effects of the neonicotinoid insecticides TMX and IMI on the physiology, antioxidant capacity, genome stability, and integrity of cultivated sunflower. The observed diverse and organ-specific plant responses strongly suggest the existence of species-specific mechanisms of neonicotinoid action that often differ among plant tissues and organs. Further studies are still needed to assess the neonicotinoid toxicity with greater resolution, which could contribute to minimization of adverse insecticide effects on crops.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-14497-y.

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**Authors' contribution** Mariyana Georgieva: Conceptualization, methodology, validation, formal analysis, investigation, writing - original draft, visualization. Georgi Bonchev: Methodology, formal analysis, writing - review and editing, resources. Grigor Zehirov: Investigation, formal analysis, validation. Vesela Vasileva: Investigation,validation. Valya Vassileva: Conceptualization, resources, writing - review and editing, supervision, project administration, funding acquisition

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**Availability of data and materials** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**Competing interests** The authors declare no competing interests.

## References

Anderson D, Yu TW, Phillips BJ, Schmezer P (1994) The effect of variousantioxidants and other modifying agents on oxygen-radical generated DNA damage in human lymphocytes in the comet assay. Mutat Res 307:261–271. https://doi.org/10.1016/0027-5107(94)90300-X

Afifi M, Lee E, Lukens L, Swanton C (2015) Thiamethoxam as a seed treatment alters the physiological response of maize (*Zea mays*) seedlings to neighbouring weeds. Pest Manag Sci 71:505–514. https://doi.org/10.1002/ps.3789

Annamalai M, Vasantha-Srinivasan P, Thanigaivel A, Muthiah C, Karthi S, Jena M, Pandi GGP, Adak T, Murugesan AG, Senthil-Nathan S (2018) Effect of thiamethoxam on growth, biomass of rice varieties and its specialized herbivore, *Scirpophaga incertulas* Walker. Physiol Mol Plant Pathol 101:146–155. https://doi.org/10.1016/j.pmpp.2017.10.009

Ansoar-Rodríguez Y, Christofoletti CA, Marcato AC, Correia JE, Bueno OC, Malaspina O, Fontanetti CS (2015) Genotoxic potential of the insecticide imidacloprid in a non-target organism (*Oreochromis niloticus*-Pisces). J Environ Prot 6:1360–1367. https://doi.org/10.4236/jep.2015.612118

Beam A, Motsinger-Reif A (2014) Beyond $IC_{50}$s: towards robust statistical methods for *in vitro* association studies. J Pharmacogen Pharmacoprot 5:1000121. https://doi.org/10.4172/2153-0645.1000121

Benzie IF, Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 299:15–27. https://doi.org/10.1016/S0076-6879(99)99005-5

Bi JL, Tuan SJ, Toscano NC (2007) Impact of greenhouse whitefly management on strawberry fruit quality. Insect Sci 14:151–156. https://doi.org/10.1111/j.1744-7917.2007.00137.x

Bianchi J, Fernandes TCC, Marin-Morales MA (2016) Induction of mitotic and chromosomal abnormalities on *Allium cepa* cells by pesticides imidacloprid and sulfentrazone and the mixture of them. Chemosphere 144:475–483. https://doi.org/10.1016/j.chemosphere.2015.09.021

Bonchev B, Pearce S, Georgiev S (2010) Retrotransposons and ethyle methanesulphonate induced genetic diversity in sphaerococcum mutant forms of hexaploid wheat and *Triticale*. Cent Eur J Biol 5:765–776. https://doi.org/10.2478/s11535-010-0072-7

Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EAD, Noome

# Accumulation of transposable elements in selfing populations of *Arabidopsis lyrata* supports the ectopic recombination model of transposon evolution

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**Key words:** *Arabidopsis lyrata*, ectopic recombination, genome evolution, mating system, outcrossing, population size, self-fertilization, transposable elements.

## Summary

- Transposable elements (TE) can constitute a large fraction of plant genomes, yet our understanding of their evolution and fitness effect is still limited. Here we tested several models of evolution that make specific predictions about differences in TE abundance between selfing and outcrossing taxa, and between small and large populations.
- We estimated TE abundance in multiple populations of North American *Arabidopsis lyrata* differing in mating system and long-term size, using transposon insertion display on several TE families.
- Selfing populations had higher TE copy numbers per individual and higher TE allele frequencies, supporting models which assume that selection against TEs acts predominantly against heterozygotes via the process of ectopic recombination. In outcrossing populations differing in long-term size, the data supported neither a model of density-regulated transposition nor a model of direct deleterious effect. Instead, the population structure of TEs revealed that outcrossing populations tended to split into western and eastern groups – as previously detected using microsatellite markers – whereas selfing populations from west and east were less differentiated. This, too, agrees with the model of ectopic recombination.
- Overall, our results suggest that TE elements are nearly neutral except for their deleterious potential to disturb meiosis in the heterozygous state.

## Introduction

Transposable elements (TEs) comprise a substantial and dynamic part of plant genomes. Their abundance ranges from 20% to 30% of the genome in species with small genomes, such as *Arabidopsis thaliana* and *Brachypodium distachyon*, to > 85% in species with large genomes, such as maize and barley (Tenaillon *et al.*, 2010). Processes directly affecting abundance include transposition, excision, recombination, selection and genetic drift (Charlesworth *et al.*, 1994; Devos *et al.*, 2002; Le Rouzic *et al.*, 2007; Dolgin & Charlesworth, 2008). But the relative importance of these processes in determining TE abundance and the mechanisms that regulate TEs are still not clearly worked out. Several evolutionary models make different predictions depending on mating system and effective population size. Variation in mating system – selfing vs outcrossing – has a profound effect on the factors predicted to influence TE abundance, such as homozygosity and effective recombination, genetic drift, and gene flow (Nordborg, 2000; Charlesworth, 2003; Wright *et al.*, 2008). Therefore, variation in mating system and effective population size represents a promising avenue for evaluating models of TE abundance. Previous comparisons of TE evolution between mating systems have focused on plant species that diverged hundreds of thousands or millions of years ago (Wright *et al.*, 2001; Tam *et al.*, 2007; Lockton & Gaut, 2010; de la Chaux *et al.*, 2012; Middleton *et al.*, 2013; Ågren *et al.*, 2014). Here, we compared closely related populations within a single species that evolved different mating systems within the past few thousand years to test predictions of alternative models of transposon evolution.

An important set of models of TE evolution is based on the frequency of ectopic recombination (Table 1, models A3 and A5). Ectopic recombination between nonhomologous sequences or, in the context of TEs, nonallelic homologous sequences, can generate harmful chromosomal rearrangements. Selection against such chromosomal rearrangements also would indirectly select against the TEs that caused them. The 'ectopic recombination model' postulates that TEs accumulate where ectopic recombination between copies is less frequent – normally in regions with low recombination rates (Langley *et al.*, 1988). The probability of ectopic recombination between TEs may be reduced in selfing taxa because of higher levels of sequence homozygosity (Montgomery *et al.*, 1991), and this should lead to higher TE abundance (Charlesworth & Charlesworth, 1995; Wright & Schoen,

*Capsella* and *Arabidopsis*, we conclude that considerable time has to pass after a shift to selfing until TE abundance declines.

In conclusion, our study demonstrated the importance of the mating system in the evolution of TEs. Selfing populations of *A. lyrata* maintain levels of TE diversity comparable to those of outcrossing populations, judging from the fraction of polymorphic loci. Copy numbers per individual and TE frequencies are higher in selfing than in outcrossing populations, and population divergence among them is reduced. Overall, patterns of TE diversity within and among selfing compared to outcrossing taxa suggest that TE evolution is strongly driven by ectopic recombination.

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## Author contributions

G.B. and Y.W. planned and designed the research; Y.W. conducted the field sampling and G.B. did the laboratory work; and G.B. and Y.W. analysed the data and wrote the manuscript.

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## References

**Abrusán G, Krambeck HJ, Junier T, Giordano J, Warburton PE. 2008.** Biased distributions and decay of long interspersed nuclear elements in the chicken genome. *Genetics* **178**: 573–581.

**Ågren JA, Wang W, Koenig D, Neuffer B, Weigel D, Wright SI. 2014.** Mating system shifts and transposable element evolution in the plant genus *Capsella*. *BMC Genomics* **15**: 602.

**Barrón MG, Fiston-Lavier AS, Petrov DA, González J. 2014.** Population genomics of transposable elements in *Drosophila*. *Annual Review of Genetics* **48**: 561–581.

**Boutin TS, Le Rouzic A, Capy P. 2012.** How does selfing affect the dynamics of selfish transposable elements? *Mobile DNA* **3**: 5.

**Charlesworth D. 2003.** Effect of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **358**: 1051–1070.

**Charlesworth B, Charlesworth D. 1983.** The population dynamics of transposable elements. *Genetical Research* **42**: 1–27.

**Charlesworth D, Charlesworth B. 1995.** Transposable elements in inbreeding and outbreeding populations. *Genetics* **140**: 415–417.

**Charlesworth B, Sniegowski P, Stephan W. 1994.** The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**: 215–220.

**de la Chaux N, Tsuchimatsu T, Shimizu KK, Wagner A. 2012.** The predominantly selfing plant *Arabidopsis thaliana* experienced a recent reduction in transposable element abundance compared to its outcrossing relative *Arabidopsis lyrata*. *Mobile DNA* **3**: 2.

**Crow JF, Kimura M. 1970.** *An introduction to population genetics theory*. Minneapolis, MN, USA: Burgess.

**Deceliere G, Charles S, Biémont C. 2005.** The dynamics of transposable elements in structured populations. *Genetics* **169**: 467–474.

**Devos KM, Brown JKM, Bennetzen JL. 2002.** Genome size reduction through illegitimate recombination counteracts genome expansion in *Arabidopsis*. *Genome Research* **12**: 1075–1079.

**Dolgin ES, Charlesworth B. 2008.** The effects of recombination rate on the distribution and abundance of transposable elements. *Genetics* **178**: 2169–2177.

**Du J, Tian Z, Hans CS, Laten HM, Cannon SB, Jackson SA, Shoemaker RC, Ma J. 2010.** Evolutionary conservation, diversity and specificity of LTR-retrotransposons in flowering plants: insights from genome-wide analysis and multi-specific comparison. *Plant Journal* **63**: 584–598.

**Durvasula A, Fulgione A, Gutaker RM, Alacakaptan SI, Flood PJ, Neto C, Tsuchimatsu T, Burbano HA, Picó FX, Alonso-Blanco C *et al.* 2017.** African genomes illuminate the early history and transition to selfing in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **114**: 5213–5218.

**Earl DA, vonHoldt BM. 2012.** STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**: 359–361.

**Evanno G, Regnaut S, Goudet J. 2005.** Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611–2620.

**Excoffier L, Smouse PE, Quattro JM. 1992.** Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

**Foxe JP, Stift M, Tedder A, Haudry A, Wrigth SI, Mable BK. 2010.** Reconstructing origins of loss of self-incompatibility and selfing in North American *Arabidopsis lyrata*: a population genetic context. *Evolution* **64**: 3495–3510.

**Griffin PC, Willi Y. 2014.** Evolutionary shifts to self-fertilisation restricted to geographic range margins in North American *Arabidopsis lyrata*. *Ecology Letters* **17**: 484–490.

**Hollister JD, Smith LM, Guo YL, Ott F, Weigel D, Gaut BS. 2011.** Transposable elements and small RNAs contribute to gene expression divergence between *Arabidopsis thaliana* and *Arabidopsis lyrata*. *Proceedings of the National Academy of Sciences, USA* **108**: 2322–2327.

**Hu TT, Pattyn P, Bakker EG, Cao J, Cheng JF, Clark RM, Fahlgren N, Fawcett JA, Grimwood J, Gundlach H *et al.* 2011.** The *Arabidopsis lyrata* genome sequence and the basis of rapid genome size change. *Nature Genetics* **43**: 476–481.

**Huttley GA, MacRae AF, Clegg MT. 1995.** Molecular evolution of the *Ac Ds* transposable-element family in pearl millet and other grasses. *Genetics* **139**: 1411–1419.

**Langley CH, Montgomery E, Hudson R, Kaplan N, Charlesworth B. 1988.** On the role of unequal exchnage in the containment of transposable element copy number. *Genetical Research* **52**: 223–235.

**Larson G, Schaetzl R. 2001.** Origin and evolution of the Great Lakes. *Journal of Great Lakes Research* **27**: 518–546.

**Le QH, Bureau T. 2004.** Prediction and quality assessment of transposon insertion display data. *BioTechniques* **36**: 222–228.

**Le Rouzic A, Boutin TS, Capy P. 2007.** Long-term evolution of transposable elements. *Proceedings of the National Academy of Sciences, USA* **104**: 19375–19380.

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# Retrotransposon-related genetic distance among inbred lines of sweet corn (*Zea mays* var. *saccharata*) and hybrid performance

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## Abstract

Heterosis is a main force underlying the hybrid seed industry in maize. Our experimental approach consists of a correlation study between retrotransposon-related genetic distances between parental inbred lines and hybrid performance. The assumption is that, at least for certain traits, heterosis results from genome rearrangements, largely related to retrotransposon insertions and/or removals. Fifteen maize inbred lines and one $F_1$ hybrid, representative of the genetic diversity among sweet corn and field corn lines were screened for polymorphism by retrotransposon microsatellite amplified polymorphism markers. DNA fingerprints served as row data for estimating genetic diversity of maize inbred lines and its correlation with the heterotic effect in their hybrids. A correlation between phenotypic and molecular distances was evident only at the level of individual inbred lines. Weak correlation between genetic distances and heterosis effect was observed for the average of all inbred lines. Phenotypic distances negatively correlated with heterosis for insertion height, diameter of the ear and number of kernel rows per ear. The relative contribution of each inbred line to heterosis in its derived hybrids was also estimated. Accordingly, we identified inbred lines with increased genetic distances that mostly add to the heterosis effect in their hybrids and that we recommend as prospective to be used in maize breeding programmes.

**Keywords:** genetic distance, heterosis, maize, molecular markers, transposable elements

## Introduction

Heterosis, or increased hybrid vigour in hybrids, has been exploited extensively in maize breeding. The heterosis effect has been assumed to depend on the level of genetic divergence between parents (Falconer, 1981). Therefore, the preliminary genetic screening of parental inbred lines is considered crucial for the prediction of superior crosses for efficient hybrid breeding (Lopes *et al.*, 2014).

In general, the more genetically distant two inbred lines are, the more likely it is that the hybrid will show increased heterosis (Flint-Garcia *et al.*, 2009). Positive correlations between genetic distances and heterosis were reported for different DNA markers such as RFLP (Godshalk *et al.*, 1990; Melchinger *et al.*, 1990; Boppenmaier *et al.*, 1992; Ajmone-Marsan *et al.*, 1998; Benchimol *et al.*, 2000), RAPD (Lanza *et al.*, 1997; Bruel *et al.*, 2006), AFLP (Ajmone-Marsan *et al.*,1998; Barbosa *et al.*, 2003) and SSR (Barbosa *et al.*, 2003; Xu *et al.*, 2004; Aguiar *et al.*, 2008; Srdić, 2011). Other studies, however, reported a low or negative correlation between both parameters (Legesse *et al.*, 2008; Fernandes *et al.*, 2015). Studying the expression profiles in maize hybrids, Stupar *et al.* (2008) showed that intraheterotic group crosses lead to higher level of heterosis when compared with inter-heterotic

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REVIEW ARTICLE

# Useful parasites: the evolutionary biology and biotechnology applications of transposable elements

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## Abstract

Transposable elements usually comprise the most abundant nongenic fraction of eukaryotic genomes. Because of their capacity to selfreplicate and to induce a wide range of mutations, transposable elements have long been considered as 'parasitic' or 'selfish'. Today, we recognize that the findings about genomic changes affected by transposable elements have considerably altered our view of the ways in which genomes evolve and work. Numerous studies have provided evidences that mobile elements have the potential to act as agents of evolution by increasing, rearranging and diversifying the genetic repertoire of their hosts. With large-scale sequencing becoming increasingly available, more and more scientists come across transposable element sequences in their data. I will provide examples that transposable elements, although having signatures of 'selfish' DNA, play a significant biological role in the maintainance of genome integrity and providing novel regulatoty networks. These features, along with the transpositional and mutagenic capacity to produce a raw genetic diversity, make the genome mobile fraction, a key player in species adaptation and microevolution. The last but not least, transposable elements stand as informative DNA markers that may complement other conventional DNA markers. Altogether, transposable elements represent a promising, but still largely unexplored research niche and deserve to be included into the agenda of molecular ecologists, evolutionary geneticists, conservation biologists and plant breeders.

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## Introduction

In the late 1940s, Barbara McClintock challenged the existing concepts of genome organization and functioning when she discovered genes prone to mobility (McClintock 1950), which were later called 'transposable elements' (TEs). Although, the existence of TEs was accepted relatively soon after by the scientific community, the biology and applications of mobile genetic elements took decades to be widely recognized. With the discovery that many of these sequences are able to selfreproduce and to induce mutations, the selfish or parasitic DNA hypothesis was born. It said that these sequences served no function in the host organism, but were simply maintained by their ability to replicate and spread copies of themselves within and even between genomes (Doolittle and Sapienza 1980; Orgel and Crick 1980). In this 'selfish' way, TEs introduce genomic conflict trying to maximize their own fitness at the expense of the host's genes (Burt and Trivers 2006; Werren 2011). Although, the TEs are primarily selfish and having deleterious effects, their activities may occasionally and stochastically confer a fitness advantage to their hosts. Nowadays, with the improvement of molecular tools for genome analysis including next generation sequencing technologies, the majority of scientists recognize that mobile elements, even behaving selfishly, play a significant biological role in the maintainance of genome integrity and diversification of the genetic repertoire of their hosts. Nevertheless, there is still an underestimation and/or lack of comprehension among scientists about the opportunity of studying TEs for resolving important research issues. The aim of this review was to highlight the significance of TEs as enhancers of genome dynamics and evolution, and to further disseminate this research issue to the biological community. First, I will provide a short overview of TEs, their distribution among eukaryotes and relation to genome size variation. Then, I will emphasize the evolutionary consequences of TEs for genome functioning and integrity through some examples in the plant and animal kingdoms. Finally, I will focus on the practical applications and perspectives of TEs for genome analysis and manipulation.

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**Keywords.** transposable elements; genome dynamics; gene regulation; molecular markers.

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# A comparative analysis of membrane intactness and genome integrity in pea, barley, and wheat in response to UVC irradiation

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**Abstract:** The maintenance of plant genome integrity plays a critical function in the processes of DNA replication, transcription, and repair. Short-wave UV radiation (UVC) is among the most harmful agents known to affect genome stability and to induce DNA damage, including double-strand breaks (DSBs). Most previous studies in plants addressed the effects of UVC radiation at the physiological level; however, little research effort has been put into genome sensitivity across different plant species. Here, we made use of the trypan blue exclusion test and neutral comet assay to assess nuclear membrane and genome integrity in response to UVC radiation in monocot and dicot plants. We found that UVC radiation substantially affects nuclear membranes and the level of DSBs in a dose-responsive manner. Furthermore, differential sensitivity across plant species was observed, with monocot plants being less vulnerable to DSBs. This allows us to speculate that plant species with larger genomes may better tolerate UVC radiation.

**Key words:** Ultraviolet radiation, genome integrity, trypan blue exclusion test, neutral comet assay, DNA double-strand breaks

## 1. Introduction

Plants are continuously exposed to solar radiation. Ultraviolet (UV) radiation, one of the components of sunlight, can be divided into three categories: long-wave UVA (315–400 nm), medium-wave UVB (280–315 nm), and short-wave UVC (100–280 nm). The ozone layer efficiently absorbs UV radiation up to about 310 nm as it shields all UVC and more than 95% of UVB. The most comprehensive data are available about the effect of UVA and UVB radiation on plants. The physiological and genetic response of plant cells to UVA radiation has been observed during stem extension, leaf development, and phototropism (Kunz et al., 2006). Most biological macromolecules are targets of UVB radiation. Alterations in important processes like photosynthesis, photomorphogenesis, seed germination, growth and development, and secondary metabolism have been observed (Mpoloka, 2008). Several studies reported an impact on membranes, phytohormones (Frohnmeyer and Staiger, 2003), and the activation of transposable elements (Qüesta et al., 2010).

UVC light is the most energetic and harmful photolytic agent that has the potential for inducing DNA damage, even at very short exposures. Similarly to UVB, the effects of UVC radiation on the plant genome can be of direct or indirect origin, detected mainly as pyrimidine dimers (adjacent thymine and cytosine), photoproducts (intrastrand cyclobutane-type pyrimidine dimers), which have the capacity to block DNA replication and transcription in plants cells. These lesions are repaired mainly by excision repair; however, incomplete processes can result in the formation of single-stranded DNA gaps sensitive to endonuclease attack (Myllyperkiö et al., 2000). Hence, DNA double-strand breaks (DSBs) also accumulate as a result of these described processes and are followed by chromosomal damage (Ma et al., 2009). In addition, UVC radiation contributes to the formation of DSBs in dividing cells most often through the production of intercellular reactive oxygen species (ROS) (Zemp et al., 2012). Several studies have reported the accumulation of endogenous DSBs caused by "cutting effects" or by the occurrence of a sufficient amount of adjacent single-strand breaks in human cells (Bogdanov et al., 1997; Tashiro, 2000). The effects of UVC irradiation on DNA depend on cell type and proliferation status, DNA repair capability, and the presence of endogenous and exogenous photosensitizers (Stapleton, 1992). Since monocotyledonous (monocot) plants have vertical patterns of leaf growth they tend to

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PRIMER

# Transposable elements and microevolutionary changes in natural populations

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## Abstract

**Transposable elements (TEs) usually represent the most abundant and dynamic fraction of genomes in almost all living organisms. The overall capacity of such 'junk DNA' to induce mutations and foster the reorganization of functional genomes suggests that TE may be of central evolutionary significance. However, to what extent TE dynamics drive and is driven by the evolutionary trajectory of host taxa remains poorly known. Further work addressing the fate of TE insertions in natural populations is necessary to shed light on their impact on microevolutionary processes. Here, we highlight methodological approaches (i.e. transposon displays and high-throughput sequencing), tracking TE insertions across large numbers of individuals and discuss their pitfalls and benefits for molecular ecology surveys.**

*Keywords*: adaptation, genome dynamics, high-throughput sequencing, retrotransposons, speciation, transposon displays

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## Introduction

Understanding the evolutionary ecology of taxa requires molecular mechanisms underlying variation and their ecological implications to be fully integrated. Our increasing knowledge of genome sequences already described a diversity of genome architectures and led to the concept of an evolutionarily dynamic genome (Lynch 2007; Koonin 2009). However, to what extent genome reorganization occurring at rapid rates influence and is influenced by the evolutionary trajectories of populations remains poorly known.

The discovery of transposable elements (TEs; i.e. DNA fragments from a few dozen of bp to 25 kb, having the ability to move within genomes; Box 1 and 2) entirely changed our appreciation of the stability of the genome (McClintock 1984). The functional and evolutionary impacts of TEs remain controversial today (e.g. Doolittle 2013). Although TEs can generally be appraised as parasites filling genomes with 'junk DNA' (Doolittle & Sapienza 1980; Orgel & Crick 1980), their biology has been primarily assessed by the phenotypic changes they induce through chromosomal rearrangements and interactions with coding sequences (Box 3). Selfish TEs indeed foster considerable variation that may influence the evolution of their host taxa (Kidwell & Lisch 2001; Biémont & Vieira 2006).

With the advent of large-scale DNA sequencing, the last decade has offered a deeper understanding of the diversity and abundance of TEs (Box 1). It is now clear that eukaryote genomes comprise much more than sets of genes, being populated with large fractions of TEs (e.g. about 65% of the human genome, de Koning *et al.* 2011; and more than 80% of the large genomes of cereals, Li *et al.* 2004). Genome biologists are accordingly inclined to emphasize on the structural and functional significance of TEs, whereas a prevalent view among evolutionary biologists seems to be that TE insertions – in their vast majority – are nearly neutral and unlikely to have a strong evolutionary impact. This may be true as host genomes evolved mechanisms repressing TE activity and given that selection in large populations may efficiently purge deleterious insertions, despite high mutational pressure (Box 2). All TEs certainly do not have a significant role, but we are still largely ignorant about their quantitative impact on evolution. Furthermore, the common practice of filtering out TE sequences from analyses makes their evolutionary consequences less probably to be assessed. Although challenging, we argue that taking TEs into consideration when surveying natural populations may shed further light on their evolutionary impact. We thus suggest directions to implement such work in the agenda of molecular ecologists.

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PRIMER

# Transposable elements and microevolutionary changes in natural populations

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## Introduction

Understanding the evolutionary ecology of taxa requires molecular mechanisms underlying variation and their ecological implications to be fully integrated. Our increasing knowledge of genome sequences already described a diversity of genome architectures and led to the concept of an evolutionarily dynamic genome (Lynch 2007; Koonin 2009). However, to what extent genome reorganization occurring at rapid rates influence and is influenced by the evolutionary trajectories of populations remains poorly known.

The discovery of transposable elements (TEs; i.e. DNA fragments from a few dozen of bp to 25 kb, having the ability to move within genomes; Box 1 and 2) entirely changed our appreciation of the stability of the genome (McClintock 1984). The functional and evolutionary impacts of TEs remain controversial today (e.g. Doolittle 2013). Although TEs can generally be appraised as parasites filling genomes with 'junk DNA' (Doolittle & Sapienza 1980; Orgel & Crick 1980), their biology has been primarily assessed by the phenotypic changes they induce through chromosomal rearrangements and interactions with coding sequences (Box 3). Selfish TEs indeed foster considerable variation that may influence the evolution of their host taxa (Kidwell & Lisch 2001; Biémont & Vieira 2006).

With the advent of large-scale DNA sequencing, the last decade has offered a deeper understanding of the diversity and abundance of TEs (Box 1). It is now clear that eukaryote genomes comprise much more than sets of genes, being populated with large fractions of TEs (e.g. about 65% of the human genome, de Koning *et al.* 2011; and more than 80% of the large genomes of cereals, Li *et al.* 2004). Genome biologists are accordingly inclined to emphasize on the structural and functional significance of TEs, whereas a prevalent view among evolutionary biologists seems to be that TE insertions – in their vast majority – are nearly neutral and unlikely to have a strong evolutionary impact. This may be true as host genomes evolved mechanisms repressing TE activity and given that selection in large populations may efficiently purge deleterious insertions, despite high mutational pressure (Box 2). All TEs certainly do not have a significant role, but we are still largely ignorant about their quantitative impact on evolution. Furthermore, the common practice of filtering out TE sequences from analyses makes their evolutionary consequences less probably to be assessed. Although challenging, we argue that taking TEs into consideration when surveying natural populations may shed further light on their evolutionary impact. We thus suggest directions to implement such work in the agenda of molecular ecologists.

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organisms without proper reference genomes. Genomic DNA can indeed be specifically enriched in fragments containing TE insertions by either PCR or sequence capture. Sequenced fragments can then be used to identify and compare flanking genomic regions among individuals at the population level. A relatively straightforward approach would be to massively sequence PCR fragments from transposon displays as otherwise achieved with AFLP (Paris & Despres 2012). Similarly, primers specific to the candidate TE families can be used together with primers annealing to the linker sequence to amplify fragments containing targeted TE insertions for the sequencing of amplicons (Witherspoon *et al.* 2010). Noticeably, PCR amplification before sequencing can be avoided by capturing fragments containing candidate TEs on custom arrays (Baillie *et al.* 2011). This approach was shown to be fairly sensitive, highlighting rare transposition events. As current high-throughput sequencing platforms yield enough data to allow for the pooling of several samples, the above-mentioned methods and forthcoming ones may be suitable for reasonably sized surveys of TE insertional patterns in natural populations of nonmodel organisms.

## Conclusions

Genomes are emerging as very dynamic entities, with their most variable fraction (i.e. TEs) probably driving changes of the overall architecture of functional genomes (Fedoroff & Bennetzen 2013). In particular, TEs seem to show bursts of activity under specific conditions that are common in the wild (Box 2) and that may translate into genome reorganization of central significance for the evolutionary ecology of the host species (Box 3). However, several interconnected processes such as selection at the TE and at the host levels may influence the evolutionary trajectories of TEs within and among taxa, and have to be better understood (Tenaillon *et al.* 2010). In addition to host-controlled transposition and TE deletion, the fate of inserted TEs is indeed determined by processes acting at the level of the host population (Le Rouzic *et al.* 2007). The distribution and accumulation of TE insertions thus depend on their effect on the host fitness and effective population size, and are thus largely determined by population features such as demography, mating system and local recombination rate that add to natural selection (Lynch 2007). Accordingly, the interplay between the environment, the host population dynamics and TE dynamics probably plays a major role in driving the evolutionary trajectories and divergence of closely related taxa. Additional work tracking TE insertions in natural populations is thus necessary to shed light on the impact of TEs (i.e. 'junk DNA') on microevolutionary processes.



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## References

Abbott R, Albach D, Ansell S *et al.* (2013) Hybridization and speciation. *Journal of Evolutionary Biology*, **26**, 229–246.

Alix K, Heslop-Harrison JS (2004) The diversity of retroelements in diploid and allotetraploid *Brassica* species. *Plant Molecular Biology*, **54**, 895–909.

Alleman M, Freeling M (1986) The Mu transposable elements of maize: evidence for transposition and copy number regulation during development. *Genetics*, **112**, 107–119.

Baillie JK, Barnett MW, Upton KR *et al.* (2011) Somatic retrotransposition alters the genetic landscape of the human brain. *Nature*, **479**, 534–537.

Baucom RS, Estill JC, Chaparro C *et al.* (2009) Exceptional diversity, nonrandom distribution, and rapid evolution of retroelements in the B73 maize genome. *PLoS Genetics*, **5**, e1000732.

Bhattacharyya M, Smith AM, Ellis THN, Hedley C, Martin C (1990) The wrinkled-seed character of pea described by Mendel is caused by a transposon-like insertion in a gene encoding starch-branching enzyme. *Cell*, **60**, 115–122.

Biémont C, Vieira C (2006) Junk DNA as an evolutionary force. *Nature*, **443**, 521–524.

Bonin A, Ehrich D, Manel S (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology*, **16**, 3737–3758.

Butelli E, Licciardello C, Zhang Y *et al.* (2012) Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. *Plant Cell*, **3**, 1242–1255.

Capy P, Gasperi G, Biémont C, Bazin C (2000) Stress and transposable elements: co-evolution or useful parasites? *Heredity*, **85**, 101–106.

Castel SE, Martienssen RA (2013) RNA interference in the nucleus: roles for small RNAs *in* transcription, epigenetics and beyond. *Nature Reviews Genetics*, **14**, 100–112.

Chang R-Y, O'Donoughue LS, Bureau TE (2001) Inter-MITE polymorphisms (IMP): a high throughput transposon-based genome mapping and fingerprinting approach. *Theoretical and Applied Genetics*, **102**, 773–781.

DeBarry JD, Liu R, Bennetzen JL (2008) Discovery and assembly of repeat family pseudomolecules from sparse genomics sequence data using the assisted automated assembler of repeat families (AAARF) algorithm. *BMC Bioinformatics*, **13**, 235.

Devos KM, Ma J, Pontaroli AC, Pratt LH, Bennetzen JL (2005) Analysis and mapping of randomly chosen bacterial artificial chromosome clones from hexaploid bread wheat. *Proceedings of National Academy of Sciences USA*, **102**, 19243–19248.

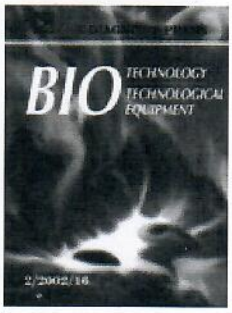
Dolgin ES, Charlesworth B (2008) The effects of recombination rate on the distribution and abundance of transposable elements. *Genetics*, **178**, 2169–2177.

Doolittle WF (2013) Is junk DNA bunk? A critique of ENCODE. *Proceedings of National Academy of Sciences USA*, **110**, 5294–5300.

Doolittle WF, Sapienza C (1980) Selfish genes, the phenotype paradigm and genome evolution. *Nature*, **284**, 601–603.

Estep MC, De Barry JD, Bennetzen JL (2013) The dynamics of LTR retrotransposon accumulation across 25 million years of panicoid grass evolution. *Heredity*, **110**, 194–204.

Fedoroff NV (2012) Transposable elements, epigenetics, and genome evolution. *Science*, **338**, 758–767.



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# Plant Tansposable Elements. A Focal Point for Future Studies of the Plant Genomes

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