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遗传不育技术在蚊媒疾病防控中的应用

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摘要: 疟疾、登革热等重大传染性蚊媒疾病严重危害人类健康,且目前缺乏有效的药物和疫苗,防治埃及伊蚊、冈比亚按蚊等媒介昆虫是控制和消除这些疾病的有效手段。化学杀虫剂的大规模使用在一定程度上控制了疾病的传播,但其抗药性和环境污染等问题也随之而来。分子生物学的飞速发展为昆虫不育技术(SIT)的更新及害虫防治提供了新的策略,由此发展起来的以释放携带显性致死基因昆虫(RIDL)为代表的一系列遗传不育技术为蚊虫种群防控提供了更加有效的选择。本文概述了遗传技术在蚊虫防控中的应用进展,包括蚊虫遗传防治的历史和策略,阐述了 RIDL 技术体系的原理,同时介绍了相关遗传控制品系和已经开展的田间释放研究,展示了遗传修饰不育技术在蚊媒疾病防治中的巨大潜力。

关键词: 蚊媒昆虫; 遗传防治; 昆虫不育技术; 释放携带显性致死基因昆虫的技术

Application of genetic pest management in the control of mosquito-borne diseases

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Abstract: Mosquito-borne diseases, such as dengue fever and malaria, are global problems and pose a serious threat to public health. An estimated 2.5 billion people live in areas at the risk of epidemic transmission. For now, no vaccines are available against the pathogens respnsible for these diseases, and the mosquito control is considered as one of the most effective ways to reduce transmission. Mass application of pesticides could reduce the mosquito population but it also brings problems like insect resistance and environmental pollution. The release of insects with dominant lethality (RIDL) technology and other genetic control systems based on the traditional sterile insect technique (SIT) provide new strategies to control disease vector mosquitos, such as *Aedes aegypti* and *Anopheles gambiae*. Those new version of genetic control methods are species-specific and environment-friendly, and now being developed and tested worldwide. Here the principle and recent progress of mosquito genetic control are reviewed. The history of mosquito SIT is introduced, and the genetic control strategies including self-limiting and self-sustaining populations are also illustrated. The development, as well as laboratory and field trials of RIDL strains are described. It is suggested that genetic control strategies such as RIDL are promising methods to fight against mosquitoes carrying human diseases.

Key words: disease vector mosquito; genetic control; sterile insect technique; release of insects carrying a dominant lethal

疟疾、登革热、丝虫病、黄热病等以蚊虫为媒介的重大传染疾病严重威胁人类的健康,蚊媒防治是控制和消除这些疾病的有效手段。传统的蚊媒防治以化学药剂为主,但抗药性以及化学药剂对环境

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的污染和生态破坏等问题日益严重。分子生物学的发展为蚊媒的防治提供了新的途径,其中以昆虫遗传修饰技术与昆虫不育技术(Sterile insect technique,SIT)(Knipling,1970)相结合发展起来的昆虫遗传修饰不育技术为害虫防治提供了新的思路。通过在媒介种群中引入携带显性致死基因或病原体抗性基因等害虫控制效应基因的人工品系,能够有效降低目标种群的数量或进行种群替代,从而阻断蚊媒对病原微生物的传播(周秀娟等,2008; Catteruccia *et al.*,2009; Klassen,2009; Wilke & Marrelli,2012)。近年来,蚊媒昆虫的遗传修饰不育技术研究发展迅速,相关品系在世界各地被广泛使用并取得了较好的效果,表明其在蚊媒疾病的防控中具有巨大的应用潜力(Catteruccia *et al.*,2009; Wilke & Marrelli,2012)。

1　蚊虫遗传防治的历史

SIT 是指通过释放辐照(Irradiation)等处理的雄虫与野生型雌虫交配,使其不育从而降低目标昆虫种群数量的一种害虫控制技术(Knipling,1970),其具有物种特异、环境友好、可工厂化生产、大面积控制等特点(Hendrichs *et al.*,2002)。SIT 在蚊媒防治中的应用已有较长的历史(表 1; Benedict & Robinson,2003)。获得不育昆虫的手段除了辐照不育以外,还包括化学不育(Chemosterilization,Ch)、胞质不亲和性(Cytoplasmic incompatibility,CI)、杂交不育(Hybrid male sterility,Hy)、减数分裂驱动(Meiotic drive)、染色体移位和重排(Chromosomal translocation and rearrangements,Tr)等,其中以辐照不育应用最为广泛(Benedict & Robinson,2003)。

表 1　传统 SIT 技术在蚊媒防治中的应用(Benedict & Robinson,2003)

Table 1　The application of traditional sterile insect technology in mosquito control (Benedict & Robinson,2003)

物种 Species	时间 Date	地点 Location	不育手段 Sterile	释放量与持续时间 No. and time released	目标 Objective	结果 Outcome	参考文献 References
埃及伊蚊 *Aedes aegypti*	1960~1961	美国佛罗里达州彭萨科拉 Pensacola, FL, USA	Ga	460 万,43 周 4.6 million, 43 w	种群压制 Population reduction	不显著 No effect could be concluded	Morlan *et al.*, 1962
	1967	美国密西西比州默里迪恩 Meridian, MS, USA	Ma	1.7 万雄蚊,2 周 17000 males, 2 w	形态学等位基因插入 Morphological allele introgression	1084 个受精卵中 2 个发育为标记个体 2 of 1084 eggs were to marked individuals	Fay & Craig, 1969
	1971	印度 Model Basti Model Basti, India	Tr	3 万雄蚊,2 周 30000 males, 4 w	持续染色体易位 Persistence of translocation in wild population	雄蚊高竞争能力,检测到持续易位 Males were competitive, persistence of translocation was observed	Rai *et al.*, 1973
	1971	印度 Shastri Nagar Shastri Nagar, India	Ma	5 万雄蚊,4 周 50000 males, 4 w	等位基因插入 Allele introgression	雄蚊高竞争能力,可见等位基因插入 Males were competitive and introgression was observed	Rai *et al.*, 1973
	1974	印度德里 Delhi, India	Ch, Tr/Sg	4.05 万,6 d, 3 次试验 40500, 6 d, 3 experiments	雄蚊交配竞争力 Male mating competitiveness	雄蚊高竞争能力 Males were competitive	Grover *et al.*, 1976b
	1974	肯尼亚蒙巴萨 Mombassa, Kenya	Tr	5.7 万,10 周 57000, 10 w	种群压制,部分不育 Population reduction, semi-sterility	部分不育,但是无长期持续染色体易位,对蛹和成虫种群无显著作用 Semi-sterility, but no long-term persistence of translocations nor a great effect on pupa and adult	McDonald *et al.*, 1977
	1975	肯尼亚蒙巴萨 Mombassa, Kenya	Tr	3.15 万,9 周 31500, 9 w	种群消减动态 Population reduction and dynamics	释放雄蚊与野生型雌蚊交配产卵孵化的杂合子后代不能存活至蛹 Hybrid progeny did not survive to pupa	Petersen *et al.*, 1977

续表 1

物种 Species	时间 Date	地点 Location	不育手段 Sterile	释放量与持续时间 No. and time released	目标 Objective	结果 Outcome	参考文献 References
白纹伊蚊 *Aedes albopictus*	1990~1991	美国伊利诺伊州东圣路易斯 E. St. Louis, IL, USA	Ma	2.1 万,3 次释放 21000, 3 releases	滞育和电泳异型酶基因插入 Diapause and rare electromorph introgression	检测到基因插入 Evidence of introgression	Hanson *et al.*, 1993
尖音库蚊 *Culex pipiens*	1970	法国蒙彼利埃巴黎圣母院 Notre Dame, near Montpellier, France	Tr	数十万,8 周 100s of thousands, 8 w	种群压制, 部分不育 Population reduction and semi- sterility	检测到持续易位和种群压制 Persistence of translocation and population reduction were observed	Cousserans & Guille, 1974; Laven *et al.*, 1972
致倦库蚊 *Culex quinquefasciatus*	1967	缅甸勃固 Okpo, Myanmar	CI	5000 头·d^{-1},9 周 5000/d, 9 w	种群消亡 Population elimination	种群消亡 Population eliminated	Laven,1967
	1968	美国佛罗里达 Seahorse Key Seahorse Key, FL, USA	Ch	2500 头·d^{-1},8 周 2500/d, 8 w	种群压制 Population reduction	不育率提高, 但卵块数稳定 Increased sterility, but plateau in the number of egg rafts	Patterson *et al.*,1970a
	1969	美国佛罗里达 Seahorse Key Seahorse Key, FL, USA	Ch	93 万,12 周 930000, 12 w	种群压制 Population reduction	种群压制或消亡部分由 SIT 造成 Population suppression/ elimination due in part to SIT	Patterson *et al.*,1970b
	1973	印度德里 Village near Delhi, India	CI+Tr/Ch	1.14 万,9/10 d,2 次试验 11400, 9/10 d, 2 experiments	雄蚊竞争交配能力 Male mating competitiveness	雄蚊高竞争能力 Males were competitive	Grover *et al.*, 1976a
	1973	印度德里 Village near Delhi, India	Tr+CI	2300 万,14 周 23 million, 14 w	种群压制 Population reduction	胞质不亲和性和染色体易位造成雄蚊不育,进而抑制目标种群 Sterility due to CI and translocation with population reduction	Curtis *et al.*, 1982
	1973	印度德里 Village near Delhi, India	Ch	3800 万,25 周 38 million, 25 w	雄性不育和种群压制 Population reduction and sterility	90%卵块不能发育, 但无明显种群压制 Up to 90% sterile egg rafts, but no clear population suppression	Rai *et al.*, 1973; Yasuno *et al.*, 1978
媒斑蚊 *Culex tarsalis*	1977	美国加利福尼亚州 CA, USA	Tr	7.6 万,4 周 76000, 4 w	种群压制 Population reduction	无明显效果 No measurable effect	Asman *et al.*, 1979
	1978	美国加利福尼亚州 CA, USA	Tr	18 万, 10 周 180000, 10 w	雄蚊竞争能力 Male competitiveness	雄蚊能与野生型雌蚊交配, 但扩散和竞争能力低 Evidence of matings, but dispersal and competitiveness were low 无明显种群压制 No evidence of population reduction	Milby *et al.*, 1980
	1979	美国加利福尼亚州 CA, USA	Ga	1.3 万 13000	种群压制 Population reduction	卵期大批量死亡比率上升 Increased egg-batch sterility	Asman *et al.*, 1980
	1981	美国加利福尼亚州 CA, USA	Ch	8.5 万, 8 周 85000, 8 w	种群压制和雄蚊交配行为 Population reduction and mating behavior	选型交配, 无明显种群压制 Assortative mating was observed and there was no population reduction	Reisen *et al.*, 1982

续表 1

物种 Species	时间 Date	地点 Location	不育手段 Sterile	释放量与持续时间 No. and time released	目标 Objective	结果 Outcome	参考文献 References
	1982	美国加利福尼亚州 CA, USA	Ma	15.9 万雌雄蚊, 6 周 159000 males and females, 6 w	红眼睛突变基因插入 Introgression of carmine eye color mutation	基因频率增加, 释放后 2 年仍保存低水平频率 Allele frequency increased and persisted at a low level for up to 2 years after releases ended	Reisen *et al.*, 1985
三带喙库蚊 *Culex tritaeniorhynchus*	1977	巴基斯坦旁遮普省 Punjab Province, Pakistan	Tr	16.7 万,2 周 167000, 2 w	雄蚊交配竞争能力 Male competitiveness	雄蚊对实验室饲养雌蚊交配竞争力高于野生型雌蚊 Males competed well for laboratory-reared females, but not wild females 外来种群补充影响了种群压制 Immigration prevented evidence of population reduction	Baker *et al.*, 1979
淡色按蚊 *Anopheles albimanus*	1972	萨尔瓦多 Lake Apastepeque Lake Apastepeque, El Salvador	Ch	440 万,22 周 4.4 million, 22 w	种群压制 Methods development and population reduction	种群消除 Population eliminated	Breeland *et al.*, 1974; Weidhaas, 1974
	1977~1979	萨尔瓦多太平洋海岸 Pacific coast, El Salvador	Ch, Ga	数亿 100s millions	种群压制 Population reduction	种群被抑制, 但意外的种群迁入影响了效果 Population suppressed in contracted release area, but unexpected immigration believed to reduce effect	Dame *et al.*, 1981
库态按蚊 *Anopheles culicifacies*	1979	巴基斯坦 Pakistan	Tr	3100	与野生型雌蚊和新释放雌蚊的交配能力 Mating with wild and released colony females	未观察到选型交配 Assortative mating did not appear to have been selected during the (difficult) colonization process 雄蚊具高竞争力 Males were competitive	Baker *et al.*, 1980
	1980	巴基斯坦 Pakistan	Ch	7500,1 周 7500, 1 w	与野生型雌蚊和新释放雌蚊的交配能力 Mating with wild and released colony females	竞争力降低, 但可见扩散、群游、交配现象 Males were less competitive, but dispersal, swarming and mating were observed	Reisen *et al.*, 1981
冈比亚按蚊 *Anopheles gambiae*	1968~1969	布基纳法索 Burkina Faso	Hy	24 万, 9 周 240000, 9 w	种群压制 Population reduction	未见卵期大批量死亡 No significant effect on egg-batch sterility 扩散能力较强, 但竞争力非常弱 Dispersal was high, but male competitiveness was poor	Davidson *et al.*,1970
四斑按蚊 *Anopheles quadrimaculatus*	1959~1960	美国佛罗里达 FL, USA	Ch	43.3 万, 48 周, 2 个释放点 433600, 48 w, 2 locations	种群压制和部分不育 Population reduction and semi-sterility	无明显种群压制, 也无部分不育 No population reduction, and no or little semi-sterility was observed	Weidhaas & Schmidt,1962
	1962~1963	美国佛罗里达 Panasoffkee Creek Panasoffkee Creek, FL, USA	Ch	5 万不育雄蚊和野生型雄蚊 50000 colony and wild males	雄蚊竞争力, 行为和不育措施 Male competitiveness, behaviour, and sterilization methods	雄蚊能成功与野生型和新释放雌蚊交配 Mating with wild and colony females was observed	Dame *et al.*, 1964

Ch.化学不育法;CI.细胞质不亲和性;Ga.γ 射线;Hy.杂交不育;Ma.仅标记(无不育处理);Sg.分离失调;Tr.染色体易位和重排。

Ch. Chemosterilization; CI. Cytoplasmic incompatibility; Ga. Gamma irradiation; Hy. Hyhybrid male sterility; Ma. Marker only; Sg. Segregation distorter; Tr. Translocation and other chromosomal rearrangements.

2 蚊子遗传防治的主要策略

2.1 种群压制

种群压制是通过降低一定区域内目标媒介蚊虫种群数量来控制蚊媒疾病的传播,该策略与化学药剂的目的类似,但是避免了杀虫剂抗性、杀伤非目标昆虫、环境污染等问题,这一策略的主要代表手段包括传统 SIT、释放携带显性致死基因昆虫的技术(Release of insects carrying a dominant lethal, RIDL)、X 或常染色体连锁的归巢内切酶基因(*Homing endonuclease gene*,*HEG*)系统、不相容昆虫技术(Incompatible insect technique, IIT)、致死—拯救(Killer-Rescue)系统、多位点混合(Multi-locus assortment, MLA)等。在种群压制策略中,必须通过周期性释放来保证效应基因在目标种群中的扩散和基因频率的提高。SIT 是蚊虫种群压制策略中应用最广泛的是害虫防治手段之一(Alphey, 2014),但该技术也存在一些难以克服的缺点,特别是 γ 射线等在诱导雄蚊不育的同时降低了其野外适合度(Scolari *et al.*, 2008; Thomas *et al.*, 2000)。而基于转座子活性和性别决定系统发展起来的 RIDL 和 fsRIDL(female-specific RIDL)(Alphey, 2014; Heinrich & Scott, 2000; Thomas *et al.*, 2000),能够释放携带条件致死基因纯合子品系(Homozygous strains)的雄蚊,该纯合子品系与野生型雌蚊交配后,雌性后代在特异致死基因作用下死亡,而雄性后代继续携带致死基因与野生型雌虫交配,引起目标种群数量的减少,连续释放后甚至能根除种群,从而阻断蚊媒对病原微生物的传播(Catteruccia *et al.*, 2009; Klassen, 2009; Wilke & Marrelli, 2012)。与传统 SIT 相比,RIDL 对蚊虫交配和野外生存适合度的损伤低,且免去了不育处理的环节,fsRIDL 甚至不需要性别筛选,这为致死基因作用时间的选择提供了更大的灵活性(Phuc *et al.*, 2007; Thomas *et al.*, 2000),大大节省了人力和物力,具有更高的遗传控制效率(Alphey *et al.*, 2011; Black *et al.*, 2011)。

HEG 驱动系统主要利用 HEG 酶能够定向识别插入在染色体上特定两段 DNA 序列之间的特点,当两条同源染色体中一条具有 *HEG* 基因时,HEG 酶将切割另一条染色体,并以前者为模板进行复制,即 homing 现象(Sinkins & Gould, 2006)。由于归巢内切酶 I-PpoI 对与 X 染色体连锁的 28S 核糖体基因的重复序列高度特异性靶定(Nolan *et al.*, 2011),当其与雄虫 X 染色体靶定时,可以在精子发生过程中切割 X 染色体导致后代雌性不存活或不产生 X 型精子;与常染色体靶定时会导致 fsRIDL;而当其与 Y 连锁则后代所有雄蚊均带有该基因,从而在减数分裂驱动下扩散(Burt, 2003; Catteruccia *et al.*, 2005; Deredec *et al.*, 2008; Windbichler *et al.*, 2011)。而 IIT 则利用了昆虫内共生菌 *Wolbachia* 的胞质不相容性(Cytoplasmic incompatibility, CI),将携带 *Wolbachia* 的雄蚊与不携带或携带不同类型 *Wolbachia* 的雌蚊交配,诱导产生胞质不相容性,其后代在胚胎期死亡;而含同种类型 *Wolbachia* 的雌雄蚊交配产生的后代可正常发育并感染沃尔巴克氏体,从而使携带该 *Wolbachia* 的品系在野生种群中迅速扩散,最终降低靶标昆虫的数量(Alphey, 2014; Hancock & Godfray, 2012; Laven, 1967; O'Connor *et al.*, 2012; Werren *et al.*, 2008; Zabalou *et al.*, 2004、2009)。

2.2 种群替代

种群替代是指将能够传播病原物的蚊虫品系替换为无法致病的品系(Alphey, 2014),其主要代表技术包括显性不足(Underdominance, UD)技术、*Wolbachia* 驱动系统、Medea 元件驱动系统、转座子(Transposons)转化系统、Y 染色体连锁的 *HEG* 驱动系统等。在种群替代策略中,效应基因能够在目标种群中自主扩散。蚊媒、病原微生物、抗性基因和基因驱动系统之间复杂的进化关系是种群替代策略研究的核心环节。目前已知的 *Wolbachia* 驱动系统、Medea 驱动系统、转座子转化系统、显性不足技术、*HEG* 等均能促进蚊媒抗性的产生(Alphey, 2014; Chen *et al.*, 2007; Moreira *et al.*, 2009)。

转座子是基因组中能自主复制和移位的 DNA 区段,广泛存在于昆虫基因组,不过大多数转座子已发生突变不表现活性。目前,转座子已被广泛应用于分子生物学研究。转座子在染色体不同位点的插入有可能导致外源基因失活或染色体重排,进而使蚊虫的适应性下降。来自粉纹夜蛾 *Trichoplusia ni* 的 *piggyBac* 转座子能特异识别 TTAA 位点,并准确切除与插入外源基因,可转入的外源基因的大小几乎不受限制,也无物种限制,是遗传修饰系统中应用最广泛的转座子之一(Fraser *et al.*, 1983; Handler, 2002),目前应用 *piggyBac* 转座子已成功获得了蚊媒的多个

遗传转化品系(Fu *et al.*, 2010; Phuc *et al.*, 2007; Wise *et al.*, 2011)。此外,研究者也将 *Hermes*(Jasinskiene *et al.*, 1998; Zhao & Eggleston, 1998)、*Minos*(Catteruccia *et al.*, 2000a、2000b)、*Mariner*(Coates *et al.*, 1998)等转座子抗性基因成功转入蚊虫的细胞系或得到遗传修饰品系。由于大部分非蚊虫来源的转座子在蚊虫中的遗传转化成功率较低,给其应用带来了一定的局限性(O'Brochta *et al.*, 2003),因此需要挖掘蚊媒自身位点特异且非连锁的转座子(Rasgon & Gould, 2005)。Arensburger *et al.* (2005)已在冈比亚按蚊 *Anopheles gambiae* 中发现了 *Herves* 转座子,但其调控机制尚不明晰。

内生菌 *Wolbachia* 能够通过增强蚊虫的自身免疫力或改变蚊虫的代谢通路等方式(Brennan *et al.*, 2008; Pan *et al.*, 2012),诱导蚊媒对病原微生物的抗性(Bian *et al.*, 2010、2013; Moreira *et al.*, 2009),抑制甚至清除病原微生物的感染(Walker *et al.*, 2011)。将抗病的蚊虫释放于靶标蚊媒种群中引起胞质不相容性,抗性 *Wolbachia* 逐步扩散到靶标种群中,进而取代易感靶标蚊媒,从根源上控制了蚊媒病的传播。基于赤拟谷盗 *Tribolium castaneum* 的 Medea 元件也能辅助蚊媒对病原微生物抗性的产生(Chen *et al.*, 2007), Medea 元件由母系特异启动子驱动的对胚胎有毒性的 RNA 或蛋白和受精卵特异的启动子驱动解毒蛋白组合在一起。由于雌性杂合子后代均具有母系遗传的毒素基因,当后代未遗传到解毒基因时将死亡,当遗传到母/父系来源的解毒基因时将存活(Alphey, 2014)。通过染色体易位等遗传操作产生的显性不足(Curtis, 1968; Davis *et al.*, 2001; Magori & Gould, 2006)和 Y 染色体连锁的 *HEG*(Alphey, 2014; Burt, 2003; Catteruccia *et al.*, 2005; Deredec *et al.*, 2008)也能驱动种群替代。不同种群替代技术的驱动效率不同,驱动效率较低的 *Wolbachia* 系统和显性不足系统需要更多的初始释放数量,而驱动效率较高的转座子系统和 *HEG* 需要的初始释放数量则相对较低(Alphey, 2014)。

3　RIDL 技术

3.1　RIDL 技术原理和现有品系

基于 tet-off 系统调控效应基因的表达是目前 RIDL 技术实现蚊虫特异性致死的最主要方式。在 tet-off 系统中,当大肠杆菌 *Escherich coli* 的转座子 Tn10 的四环素阻遏因子(tetracycline repressor, tetR)与四环素结合时,tetR 不能阻抑四环素抗性操纵子(tetracycline-resistance operon, tetO),因此下游转录不受抑制。将 tetR 的部分序列与单纯疱疹病毒 VP16 的转录活性区段组合为四环素转录激活因子(tetracycline transcriptional activator, tTA),tTA 与性别/组织/发育阶段特异性启动子构建为 tet-off 驱动载体,tetO 与 CMV 启动子构成四环素响应元件(Tetracycline response element, TRE),TRE 与效应基因组合成效应载体,进而组建为完整的 tet-off 表达系统(Gossen & Bujard, 1992)。在缺乏四环素时,tTA 与 tetO 结合引发效应基因表达;但在饲养条件存在四环素时,tTA 与四环素结合而不与 tetO 结合,无法激活下游效应基因的表达。

通过特定遗传标记筛选转化品系是 RIDL 构建过程中的关键步骤,利用不同启动子驱动荧光标记是目前的常用手段。优良的启动子—荧光基因表达模式能够大大降低转化筛选的难度,在提高 RIDL 品系构建效率的同时,有助于释放品系的后期监测。组成型启动子通常具有表达强度高、表达周期长和表达面积大的优点,英国 Oxitec 公司采用组成型启动子 *Hr5-IE1* 构建了性能良好的遗传荧光标记,全身表达 *DsRed2* 或 *GFP* 的埃及伊蚊 *Aedes aegypti* 幼虫在荧光滤镜下清晰可见(图 1A);而表达 *Hr5IE1*-DsRed2 的幼虫肛乳头呈现出斑点荧光模式,主要是来自核位点的荧光信号(图 1B)。此外,3*xP3* 启动子也常常用于构建蚊子的遗传荧光标记,以驱动如 *AmCyan*(图 1C)或 *DsRed*(图 1D)荧光基因在埃及伊蚊光学神经中的表达。

目前开发的蚊子 RIDL 品系主要来自 Oxitec 公司,目标物种包括埃及伊蚊、白纹伊蚊 *Aedes albopictus* 和冈比亚按蚊等,有的品系已经进入田间释放阶段(表 2)。Phuc *et al.*(2007)通过模型研究发现,由于存在密度依赖效应,携带晚期表达效应基因的 RIDL 品系与早期品系相比,不仅能够大大减少释放的初始虫源数量,而且能更快地达到控制种群的目的,具有更好的防治效果;通过构建埃及伊蚊 RIDL 品系 LA513A(OX513A)进行验证,在此系统中 tTA 通过反复结合 tetO 而不断积累,最终达到致死剂量;由于 tTA 既是转录激活因子,也是效应基因,该体系被称作单元件系统(Gong *et al.*, 2005)。此外,Fu *et al.*(2010)将雌蚊飞行肌特异性

启动子 *AeAct*-4(Muñoz *et al.*,2004)与 tTA 元件连接构建 tet-off 驱动载体,将细胞凋亡基因 *Nipp*1*Dm* 和 *michelob_x* 与 TRE 连接构建效应载体,将驱动品系 OX3545 分别与效应品系 OX3547 和 OX3582 杂交,获得的雌性后代在四环素缺乏时引发效应基因在飞行肌的特异性表达,从而丧失了飞行能力成为无翅型,比例高达 65.8%~98.3%,而雄蚊则不受影响;该体系既需要表达 tTA 的驱动载体,也需要表达致死基因的效应载体,因此又称作双元件系统(Alphey *et al.*,2008)。同时,Fu *et al.*(2010)利用 *AeAct*-4 启动子构建了单元件系统并得到了 OX3604C 品系,当不存在四环素时,过量表达的 tTA 导致飞行肌细胞凋亡,后代雌蚊几乎全部无翅,而雄蚊则不受影响。飞行能力对蚊子营养获取、交配及逃生等至关重要(Labbé *et al.*,2012),因此 OX3604C 实质上等同于基于 tet-off 调控下的雌性特异致死品系。Wise *et al.*(2011)调查了埃及伊蚊 OX3604C 品系雄蚊用于 SIT 的潜力,实验室条件下当以遗传修饰雄蚊:野生型雄蚊=(8.5~10):1,每周释放 1 次时,10~20 周便可压制野生型蚊虫。Labbé *et al.*(2012)采用白纹伊蚊的 *AealbAct*-4 启动子构建了 RIDL 品系 OX4358 并取得了与 OX3604C 近似的结果,表明 *Actin*-4 启动子的雌性飞行肌特异活性可能在不同蚊子种类中保守。

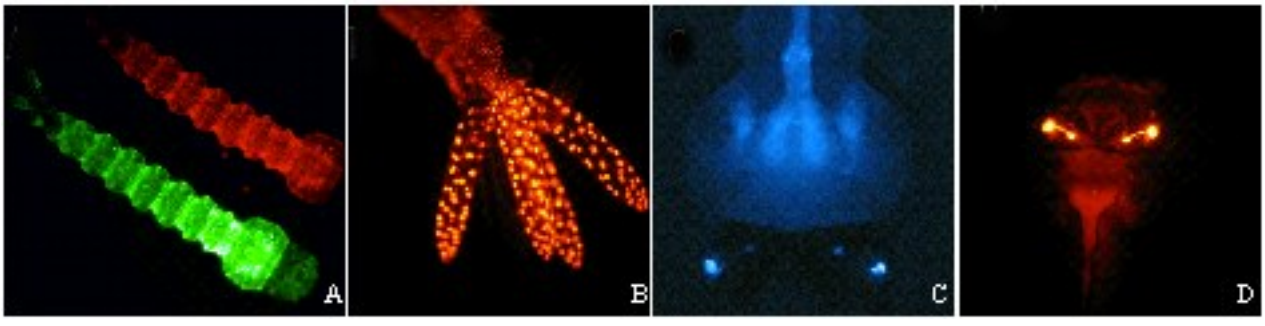

图 1 埃及伊蚊遗传修饰品系中不同遗传标记的荧光模式(图片由英国 Oxitec 公司的 Luke Alphey 博士提供)

Fig.1 Different fluorescence patterns in genetically modified strains of *Aedes aegypti* (photo courtesy from Dr. Luke Alphey, Oxitec Limited, UK)

A:*Hr*5-*IE*1 启动子驱动 *DsRed*2 和 *GFP* 在幼虫中的表达;B:幼虫肛乳头表达 *Hr*5*IE*1-*DsRed*2,斑点荧光模式来自核位点的荧光信号;C:3*xP*3 启动子驱动 *AmCyan* 在 Keele-2 品系光学神经中的表达(Nimmo *et al.*,2006);D:3*xP*3 启动子驱动 *DsRed* 在 OX3576 品系光学神经中的表达(Fu *et al.*,2010)。

A: *Hr*5-*IE*1 promoter drives *DsRed*2 and *GFP* in larvae; B: Anal papillae of the larvae expressing *Hr*5*IE*1-*DsRed*2, with a nuclear localisation signal giving the spotty appearance; C: The 3*xP*3 promoter drives *AmCyan* expression in the optic nerve in Oxitec Keele-2 strain (Nimmo *et al.*, 2006); D: The 3*xP*3 promoter drives *DsRed* expression in the optic nerve in OX3576 strain (Fu *et al.*,2010).

表 2 已经报道的蚊虫 RIDL 品系

Table 2 A list of the reported release of insects with dominant lethality (RIDL) strains in various species of mosquitoes

种类 Species	表型 Phenotype	品系 Strains	标记 Marker	转座子 Transposons	启动子 Promoter	致死基因 Lethal gene	参考文献 References
埃及伊蚊 *Aedes aegypti*	条件致死 Repressible lethality	OX513A (LA513)	*DsRed*	*piggyBac*	*Act*5*C*	*tTA*	Phuc *et al.*,2007
		LA882			*IE*-2		
	雌蚊特异致死 Female-specific flightless	OX3545 OX3547	*DsRed*	*piggyBac*	*AeAct*-4	*Nipp*1*Dm*	Fu *et al.*,2010; Wise *et al.*,2011
		OX3545 OX3582				*michelob_x*	
		OX3604C				*tTA*	
		OX3688	*AmCyan*	*piggyBac*	*AeAct*-4	*tTA*	Labbé *et al.*,2012
		OX4358			*AealbAct*-4		
白纹伊蚊 *Aedes albopictus*	雌蚊特异致死 Female-specific flightless	OX3688	*AmCyan*	*piggyBac*	*AeAct*-4	*tTA*	Labbé *et al.*,2012
		OX4358			*AealbAct*-4		
冈比亚按蚊 *Anopheles gambiae*	雌蚊特异胚胎期致死 Female-specific embryonic lethality	β2Ppo	*eGFP* *DsRed*	*piggyBac*	*β2 tubulin*	*HEG*	Galizi *et al.*,2014; Windbichler *et al.*, 2008

此外,RIDL 品系在冈比亚按蚊的种群遗传控制中也取得了理想进展。Windbichler *et al.*(2008)采用在雄蚊睾丸精子发生时特异表达的 β2 *tubulin*(Catteruccia *et al.*,2005)启动子驱动效应基因 *HEG* 的表达,导致后代雌蚊在胚胎期死亡,大幅度改变了后代的性别构成,使目标种群无法继续繁衍。由于 HEG 蛋白与 X 染色体连锁的 28S 核糖体基因的重复序列高度特异靶定,在 β2 *tubulin* 驱动下在雄蚊精子发生时切割 X 染色体,当导入胚胎时还能切割母系来源的 X 染色体,这种雄蚊与野生型雌蚊交配后导致后代雌蚊在胚胎期死亡,产生的后代几乎全为携带效应基因的雄蚊(Windbichler *et al.*,2008)。Galizi *et al.*(2014)发现应用该技术的雄蚊与野生型雌蚊交配后产生的子代 95%以上为雄性,且雄蚊的生育能力未受明显影响,到第 6 代时蚊虫种群因缺少雌性而无法繁衍,为有效控制疟疾等传染病的传播提供了更为高效、经济的方法。

3.2　田间试验

研究遗传修饰不育技术的最终目标是将培育的 RIDL 品系释放于野外替代自然界中如埃及伊蚊、冈比亚按蚊等重大传染性疾病的传播媒介,从而从根本上阻断蚊媒疾病的传播,而 RIDL 品系能否成功压制野外种群的一个重要特征在于其将携带的显性效应基因向目标种群传递扩散的能力及稳定性(Scott *et al.*,2002)。RIDL 品系在自然界中的生存(如存活率、寿命、羽化率等)、繁殖(交配竞争力等)、扩散(飞行能力等)的适应性和竞争力是反映效应基因传递能力(Massonnet-Bruneel *et al.*,2013)的重要指标。研究表明,遗传操作中转座子的遗传转化、启动子和标记基因的表达以及在饲养时为获得纯合品系采用的近亲交配等带来的自然选择压力,有可能对遗传修饰品系的适应性产生影响(Catteruccia *et al.*,2003; Irvin *et al.*,2004; Marrelli *et al.*,2006; Massonnet-Bruneel *et al.*,2013)。目前应用最多的 OX513A 品系,采用的是组成型启动子 *Act5C*,其对适合度的影响可能比组织特异性启动子更大(Massonnet-Bruneel *et al.*,2013)。因此,必须在实验室和田间开放条件下对 RIDL 品系的适合度及其释放策略进行详细调查。已有多个国家围绕该方面开展了广泛研究,并提出了 RIDL 品系的释放前标准(Benedict & Robinson,2003; Yakob *et al.*,2008)。已经报道的室内研究结果并不完全一致,但基本可以得出如下结论:RIDL 品系在生活史特性、繁殖和扩散能力等方面有一定的下降,但是与野生型相比不存在较大的差异;这可以通过进一步优化饲养体系和方法得到改善,最优释放策略的制定也有助于获得理想的控制效果(表 3)。

目前田间开放条件的释放研究主要集中于埃及伊蚊的 OX513A 品系。Harris *et al.*(2011)于 2009 年在 Grand Cayman 地区首次进行了 OX513A 雄蚊的田间释放,结果表明,释放的雄蚊能成功与野外雌蚊交配并使其受精,具有与野生雄蚊相当的繁殖能力,释放后收集的卵孵化出的幼虫能检测到荧光,且随时间推移比率逐渐提高,反映了 RIDL 在野外逐步降低野生型种群的过程。此外,模型分析发现,当田间野生型和释放 RIDL 品系交配比率达 13%~57%时才能成功抑制目标种群。因此,Harris *et al.*(2012)于 2010 进行了第 2 次释放研究,释放 4~6 周后野生型种群受到抑制,11 周时 RIDL∶野生雄蚊高达 25.2∶1,卵带荧光比率为 88%,表明 OX513A 品系成功实现了对野生型的压制。Lacroix *et al.*(2012)在马来西亚 Pahang 地区也进行了 OX513A 和野生型实验室品系的释放,结果表明,RIDL 品系不会对人类健康和环境产生不利影响,OX513A 和野生型寿命相当,虽然飞行能力有一定减弱但释放措施的改善能提高其应用效果。此外,Alphey (2014)在巴西应用 OX513A 品系也成功地实现了对 2 个目标种群的压制,并且后续的大规模释放研究还在继续开展。

4　总结

遗传不育技术成功地将昆虫不育技术与新兴的遗传修饰技术相结合,成为物种特异、环境安全、科学高效的有害生物治理手段,具有传统防治方法难以比拟的优势。目前,已经开发了包括埃及伊蚊、白纹伊蚊、冈比亚按蚊在内的众多主要媒介蚊虫的特异性致死或无翅型 RIDL 品系,并进行了一系列适应性测试和安全性评估,针对埃及伊蚊的 OX513A 品系已经实现了田间释放,验证了 RIDL 技术在防控蚊媒疾病中的可行性。种群的释放策略对蚊媒种群的遗传控制效果具有重要的影响,合理评估遗传修饰品系的适应性、扩散能力和生殖能力,构建基于昆虫学、流行病学、生物经济学的数学模型,进而优化释放比例,合理布局释放点、释放频率、持续时间等是达到最优释放策略的必要程序

(Alphey *et al.*,2011; Atkinson *et al.*,2007)。此外,遗传控制技术与化学防治、生物防治等多种控制措施联合应用,将能更加有效地控制和阻断疟疾、登革热等重大蚊媒疾病的发生和传播。

表 3 RIDL 品系适应性的室内研究结果

Table 3 Reproted fitness of mosquito strains carrying genes with dominant lethality under laboratory conditions

品系 Strain	体型 Body size	繁殖 Reproduction	生活史特征 Life span parameters	飞行 Flight	生理指标 Physiological parameters	参考文献 References
OX513A	较野生型小或无影响 Smaller/no 幼虫密度增大时,体型变小 Reduce (increase larval density)	无明显差异 Comparable	幼虫成活率降低 5% Larval survival: 5% lower 成虫寿命降低或无影响;增大幼虫密度成虫寿命降低 Adult longevity: no/reduced, decrease (increase larval density) 化蛹提前 1 d,增大幼虫密度时推迟 1 d Pupation: one day sooner, delay (increase larval density) 基本生活史参数和发育速率无影响 Basic life-history and growth rate: no	飞行距离和速度降低 Less distance; slower	糖原/碳水化合物和油脂含量接近 Glycogen/sugar/lipid: similar 对杀虫剂敏感性不受影响 Susceptibility to insecticides: similar	Bargielowski *et al.*,2011a、2011b、2012; Lee *et al.*, 2009; Massonnet-Bruneel *et al.*, 2013; Nazni *et al.*,2009
OX513A 半田间试验 Semi-field	无影响 No	无明显差异 Comparable	–	–	–	Lee *et al.*,2013
OX3604C	无影响 No	–	–	飞行距离接近,但时间减少 Similar distance; less time 四环素添加与否不影响 Tetracycline: no effect	糖原/碳水化合物含量接近;但油脂含量高于野生型,添加四环素也能提高油脂含量 Glycogen/sugar: similar; lipid: higher, increase (add tetracycline)	Bargielowski *et al.*,2012

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