THE
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GROUP

Captive Propagation and Release Plan for Quino Checkerspot Butterfly (*Euphydryas editha quino*)

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Table of Contents

1 Introduction 1
2 Background 2
  2.1 Species Ecology 2
  2.2 Captive Propagation Program Progress 1997–2010 2
3 Purpose of the Propagation and Release Program 4
  3.1 Purpose of Captive Propagation 4
  3.2 Purpose of Release Program 6
4 Strategic and Managerial Considerations 8
  4.1 Precedence of Natural Populations 8
  4.2 Possible Adverse Effects of Reintroduction 8
  4.3 Conservation Agreements, Safe Harbors, and Easements 9
  4.4 NEPA and CEQA 10
  4.5 Site-specific Release Plans 10
  4.6 Timeframe for Release and Management 11
5 Genetic and Demographic Considerations 12
  5.1 Choice of Founders for New Populations 12
  5.2 Life Stage and Number of Founders for New Populations 14
  5.3 Appropriate Range 15
6 Ecological Considerations 15
  6.1 Resources 16
    6.1.1 Space 16
    6.1.2 Microtopography 17
    6.1.3 Nectar Sources 17
    6.1.4 Larval Hostplants 17
  6.2 Malentities 17
  6.3 Mates 18
  6.4 Predators, Parasites, and Disease 18
7 Technical Considerations 18
  7.1 Captive Rearing 18
    7.1.1 Adults and Eggs 19
    7.1.2 Larvae and Diapause 19
    7.1.3 Methods to Reduce Risk 19
      7.1.3.1 Genetic Contamination 20
      7.1.3.2 Disease 20
      7.1.3.3 Parasites 21
      7.1.3.4 Genetic Drift/Selection 22
  7.2 Release Protocols 22
  7.3 Post-Release Monitoring 23
8 Adaptive Management Structure 24
  8.1 Decision analysis framework 25
    8.1.1 Establish need for release 25
    8.1.2 Rapid overall assessment 26
    8.1.3 Identify multidisciplinary team 26

8.1.4 Conduct habitat assessment....26
8.1.5 Identify donor population....26
8.1.6 Identify any needed rearing or breeding resources....27
8.1.7 Assess socioeconomic and legal requirements....27
8.1.8 Write site-specific release plan and secure administrative permissions....27
8.1.9 Evaluate release-stock for suitability....27
8.1.10 Transport and release....28
8.1.11 Post-release documentation and monitoring....28
8.2 Release objectives....29
8.3 Assessment and endpoints....31
9 Literature Cited....31

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1 Introduction

Quino checkerspot butterfly (*Euphydryas editha quino*) was listed as an endangered species on January 16, 1997 (63 Federal Register 2313–2322). Since that time, a captive propagation program has been established to guard against extinction of the species in the wild and to provide stock for release within the species former range. Although this program has successfully produced new generations of butterflies, the program as a whole lacks a long-term plan and decision making framework to evaluate which individuals should be released at which locations to maximize the probably of survival of the species at release sites. Genetic diversity is a serious concern for populations of butterflies kept in captivity (Crone et al. 2007; Snyder et al. 1996) and therefore operating procedures and record keeping are necessary to minimize selection and drift in captivity. This document assesses the current status of the captive rearing program and provides a framework to guide future actions involving reintroduction, augmentation and captive propagation of this taxon.

Any captive propagation protocol and release plan for Quino checkerspot butterfly must ensure that the captive propagation program is consistent with the USFWS Policy Regarding Controlled Propagation of Species Listed Under the ESA (65 Federal Register 56916). This plan addresses the 14 criteria in the captive propagation policy and includes:

- identification of measurable objectives and milestones for the program,
- a clear endpoint for the program,
- general operating procedures for rearing and release,
- guidance for data collection and management,
- a decision making structure to guide reintroduction strategies, and
- a post-release monitoring strategy.

2 Background

2.1 Species Ecology

The Quino checkerspot butterfly, *Euphydryas editha quino*, is a southern California subspecies of Edith's checkerspot, *Euphydryas editha.* Historically distributed widely from Los Angeles to Baja California, the subspecies is now restricted to populations in Baja, San Diego and Riverside counties. Quino was listed as an endangered subspecies in 1997 due to increasing threats of habitat loss, fragmentation and degradation (Mattoni et al. 1997, U.S. Fish and Wildlife Service 2003, 2009a, 2009b).

Quino checkerspots are univoltine with a flight period typically lasting from February to April. Females are known to deposit eggs on the following primary host plants: *Plantago erecta, Plantago patagonica, Collinsia concolor, and Anterrhinum coulteranium*. Larvae are also known to feed on *Cordylanthus rigidus and Castilleja exserta*, but these species are not known to be primary host plants for Quino (74 FR 28776 – Revised critical habitat rule). Quino adults use a variety of small annuals for nectar sources including species of *Lasthenia*, *Linanthus*, *Gilia*, *Salvia* and *Cryptantha* (Mattoni et al. 1997). Land development, invasive plant species and shifting management practices (e.g. grazing and fire) have all contributed to Quino population declines and extinctions through the degradation of these native plant communities on which Quino relies.

2.2 Captive Propagation Program Progress 1997–2010

The U.S. Fish and Wildlife Service contracted for captive propagation of Quino checkerspot butterfly that began shortly after the species was listed in 1997. Dr. Gordon Pratt, a researcher at the University of California, Riverside, initiated the program with collections of female butterflies in March, 1997 in Jacumba, southern San Diego County, and in April, 1997 at Big Oak Mountain in southwestern Riverside County (Pratt et al. 2000).

Pratt successfully obtained eggs and larvae in captivity. The female from Jacumba laid 300–400 eggs, with 135 larvae surviving to diapause, while the Big Oak Mountain female laid 52 eggs with 39 surviving to diapause (Pratt & Emmel 1998; Pratt et al. 2000). After diapause, the offspring of the founders were mated with the two locations segregated (Pratt & Emmel 1998; Pratt et al. 2000).

The captive propagation program over the subsequent years was directed toward maintaining captive populations of Quino checkerspots from five different areas of the species' range. Over the years, varying numbers of individuals were successfully maintained for each geographic line. At several points in the program females from a location were taken to the field and mated with wild males (Table 1). Over the 10 years of the program larvae were provided to other researchers for research purposes (Pratt & Stouthamer 2002).

Table 1. Number of females collected and number mated with wild males (in parenthesis) listed.

	SE San Diego Jacumba	S Riverside Wilson Valley/Big Oak Mountain	S Riverside/N San Diego Counties Anza/Silverado	SW San Diego Marron Valley	SW Riverside Lake Skinner
1997	1(0)	1(0)			
1998				1(0)	
2002				3(0)	10(0)
2004	2(0)		3(0)	0(6)	1(4)
2005	1(1)		l.c.		2(10)

Originally five regions were included in the captive breeding program: 1) Lake Skinner and west in Riverside County, 2) east of Wilson Valley to high elevations in the western San Jacinto Mountains, 3) southwestern San Diego County, including Jacumba, 4) southeastern San Diego County, including Marron Valley, and 5) Wilson Valley north to Brown Canyon (Table 2). The region east of Wilson Valley was dropped from the program in the mid-2000s because multiple field visits during years of adequate rainfall yielded no butterflies (Pratt undated). These regions correspond with the recovery units designated in the Recovery Plan for the species (U.S. Fish and Wildlife Service 2003).

The captive rearing program has demonstrated the techniques necessary to produce butterflies in the laboratory and has facilitated research on larval ecology (Pratt & Emmel 2009). Methods for propagation have been documented on the website www.quinocheckerspot.com (Pratt undated). The program focused on perfection of rearing techniques, investigation of larval ecology, and maintenance of genetic diversity. Detailed records to document the lineage of each specimen are not easily accessible.

The captive breeding program has not been used to provide butterflies for the purpose of reintroduction to restored or existing but unoccupied habitat.

Table 2. Number of larvae produced in captive propagation program (Pratt 2004, 2005, 2007; Pratt & Emmel 1998; Pratt et al. 2000; Pratt & Stouthamer 2002). The numbers of larvae at the end of the year are listed, whether they were larvae of the year or from previous years.

	SE San Diego Jacumba	S Riverside Wilson Valley/Big Oak Mountain	S Riverside/N San Diego Counties Anza/Silverado	SW San Diego Marron Valley	SW Riverside Lake Skinner
1997	135	39			
1998				65	
1999					
2000					
2001		133		262	229
2002	198	472	122	974	4411
2003	1				
2004	620	0	450	1420	595
2005	234	0	370	1725	2110
2006	276	0	610	1327	1787
2007	26	0	395	2950	8775
2008	4	0	515	1239	3675
2009	0	0	20	437	3898
2010	0	0	121	67	2106

3 Purpose of the Propagation and Release Program

3.1 Purpose of Captive Propagation

Three main benefits could result from captive propagation for Quino checkerspot butterfly: a refugium population as insurance against extinction in the wild, research on the ecology of the species, and to provide material for the release of individuals back into the wild for reintroduction or augmentation. The existing captive propagation program has functioned as a refugium for a few of the recovery units and resulting in research on rearing techniques and species ecology (e.g., Pratt and Emmel 2009). The Service, however, no longer believes that a refugium is necessary to prevent extinction of the subspecies (because the known occurrence are substantially more than previously known) (U.S. Fish and Wildlife Service 2009a). Refugia may be necessary, however, for local "habitat-based population distributions" or "occurrence complexes" that are under threat of extirpation.

Going forward, the captive propagation should be tailored to achieve the following objectives:

1. Maintain a captive population of Quino checkerspots from the habitat-based population distributions at highest risk of extirpation.
2. Research and report findings on the ecology, genetics, and husbandry of Quino checkerspot.
3. Provide short or long-term captive care of Quino checkerspots to increase numbers for the purpose of release.

This program can be deemed successful if it maintains a genetically stable population from each of the recovery units, is able to provide adequate numbers of butterflies or larvae for the purpose of release where it is deemed necessary, and reports these activities accurately and completely.

The Warm Springs Creek Core habitat-based population distribution is currently identified as being at high risk of extirpation (U.S. Fish and Wildlife Service 2009a) and therefore should be a candidate for captive breeding as a refugium. Any number of other occurrence complexes face high threat levels (U.S. Fish and Wildlife Service 2009a: Table 1) and could be candidates for establishing a captive population as a refugium. With the conclusion that the larger occurrence complexes are stable in the short run, refocusing effort on those areas that are both isolated from these stable core distributions and at risk of extirpation is a logical next step for ongoing captive breeding efforts.

3.2 Purpose of Release Program

Release of butterflies at a location can be done for several reasons. These include augmentation, reintroduction within the known range of the species, and establishment outside the known range of the species. Each of these actions should be practiced only when the factors that contributed to the target population decline/extirpation (or absence) are adequately understood to the extent that there is a reasonable expectation that the release will be successful.

In the event of a severe population decline, some populations would benefit from augmentation either from captive-reared or translocated butterflies. Augmentation efforts need to consider possible detrimental impacts (e.g. disease transmission) of reintroduction on present butterflies. Because of the explosive growth capacity of checkerspots (Boggs et al. 2006), augmentation is unlikely to be necessary under most circumstances. However, when population densities are too low Allee effects can be apparent in some butterfly metapopulations (Kuussaari et al. 1998) and augmentation should be considered in this context.

The main focus of a release program should be reintroduction within the range of the species. This takes the form of release into existing habitat that is assessed to be suitable but not occupied but presumably was occupied in the past.

As the climate changes, it is possible that suitable habitat may be created where it previously did not exist. For example, under warming scenarios Quino checkerspot habitat is projected to shift upslope (Preston et al. 2008). In such scenarios, release to a site that was not previously occupied might be contemplated. The most likely scenario for Quino checkerspots is a shift in elevation within the current geographic footprint of the range, since movement along the borders, especially to the north, is complicated by boundaries with other subspecies and extensive urban development.

The purpose of the release program therefore should have the following objectives (in ranked order).

1. Reintroduce Quino checkerspots to existing or restored habitat within the current species range that are not currently occupied.
2. Augment existing populations that are either near extirpation, or are recently extirpated and absence cannot be assumed.
3. Create new populations at locations that are not previously known to be within the subspecies range, but are now assessed to be suitable and likely to remain so.

Establishment of new populations of a butterfly is difficult and subject to setbacks. Reintroduced individuals are very unlikely to establish populations which will persist indefinitely without further intervention. Rather, release of butterflies to suitable habitats, in conjunction with management of those habitats, is likely to be part of any ongoing management plan for the species. The population dynamics of the species are naturally prone to extirpation and colonization (Mattoni et al. 1997) and this means that even a "successful" reintroduction may not persist more than a few years or a decade. Consequently, the goal should be to establish populations wherever appropriate habitat is available and release is feasible, recognizing that continuous occupancy into the future at any particular release site is unlikely at best. A successful release is any time a full reproductive cycle of the butterfly can be observed that does not depend on the released material. The establishment of a stable metapopulation would determine the successful completion of the release program.

To achieve the purposes articulated here for propagation and release of Quino checkerspot, many factors must be taken into consideration. The sections that follow articulate the strategic, managerial, genetic, demographic, and technical considerations necessary to implement this plan. The next section then outlines the decision making process for deciding upon a release or propagation project and the final section evaluates this plan's compliance with the Service's captive propagation policy.

4 Strategic and Managerial Considerations

4.1 Precedence of Natural Populations

The purpose of endangered species conservation is not to prop up wild populations through the continuous provision of captive bred stock. Nor is it to prioritize reintroduction and establishment of populations as mitigation for loss of natural populations. As a general principle, the protection, management, and enhancement of existing Quino checkerspot populations should be the top priority for conservation efforts. The success rate for establishing Quino checkerspot butterfly populations is unknown and reintroduction should be seen as a pathway to assist recovery but new and on-going efforts to conserve existing populations should be continued and coordinated with reintroduction efforts.

4.2 Possible Adverse Effects of Reintroduction

The consequences of a reintroduction must be assessed from the perspective of the donor population and the target population. Any removal of individuals from a donor population could pose a risk to the persistence of that donor population. For translocation efforts this should be of particular concern. However, for Quino checkerspots, removal of individuals from a donor population is not likely to be detrimental as long as collection is not excessive (e.g. below 5% of the total donor population). As explained elsewhere here, there should also be a reasonable expectation of success and assurances should be made that factors that contributed to population decline or absence have been addressed. Otherwise, even if there is not an appreciable impact on the donor population significant and unnecessary costs could be incurred.

Adverse impacts of reintroductions on wild butterfly populations could occur if the reintroduction decreases population growth rate through the introduction of disease or deleterious alleles (Crone et al. 2007). The extent to which reintroductions may harm wild individuals may depend on the length of time in captivity. Captive "rearing" of butterflies - bringing mated females into captivity, raising their offspring to adulthood and releasing those adults into the wild – may be less risky than captive "breeding" or keeping multiple generations of butterflies in captivity (Crone et al. 2007). However, even captive reared butterflies may differ from their wild counterparts morphologically though the consequences of these differences and captive rearing to target populations generally are little known (Schultz et al. 2009).

For target sites that are currently unoccupied there is clearly no concern about detrimental effects of long-term captivity on the non-existent population. Within the larger metapopulation context, however, there could potentially be consequences of captive-bred butterflies on neighboring population growth rates should they come into contact. So even in the case of reintroductions into unoccupied sites, it is likely still advantageous to minimize time in captivity.

Notwithstanding these potential adverse effects of reintroduction efforts, the steps to reduce or eliminate them described below will ensure that such potential impacts are not significant and should not hinder efforts to expand the number of sites occupied by Quino checkerspot.

4.3 Conservation Agreements, Safe Harbors, and Easements

Any reintroduction site should be protected by a legal agreement for some significant period of time into the future before any release is considered. The effort required to undertake a release is too significant to allow a change of ownership or owner priorities to jeopardize a reintroduction effort. Such protections can range from ownership by the Fish and Wildlife Service (Refuge system), permanent conservation easements, ownership by government agency or private conservation organization that identifies maintenance of the species as the primary management objective for the site, Safe Harbors Agreements that secure conservation management of a site for a significant period of time, and other habitat conservation plans and conservation agreements. Safe Harbor Agreements can be particularly important in assuring neighbors of a property that reintroduction will not increase their own regulatory burden. Because of the metapopulation dynamics of the species, a population that is maintained for some period of time can be important to regional dynamics and consequently is better than no population. Permanent protection of a site is therefore not always necessary, but at least a 10-year commitment should be a minimum, with priority for releases going to permanently protected sites.

4.4 NEPA and CEQA

Any captive propagation or release project for Quino checkerspot will require evaluation under the National Environmental Policy Act if it takes place on federal land, uses federal funds, or requires a federal permit. Furthermore, any project that requires a discretionary action by a state or local jurisdiction may also be subject to the California Environmental Quality Act. For such projects, the lead agency (mostly likely FWS) will need to develop an Environmental Assessment (EA) and/or other appropriate documentation that describes the need for the project, account for any potential environmental impacts, and consider alternatives to the proposed project. Reintroductions qualify for a Categorical Exclusion under NEPA but if surrounding landowners may be affected, an EA is necessary. This process may involve public hearings and will guarantee the opportunity for public comment from stakeholders and any interested individuals or organizations. Those developing propagation or release projects for Quino checkerspot should consult the FWS early in the process to ensure consistency with NEPA/CEQA obligations.

4.5 Site-specific Release Plans

Each site where Quino checkerspot is to be released should have a site-specific Release Plan in place that has been adopted by the property manager to guide the project. Because recent surveys have identified additional larval foodplants and habitat conditions (Pratt et al. 2001), it is not possible to establish a generic plan that would apply across the range of the species. The elements of a site-specific plan are outlined below (Table 3).

Table 3. Elements of site-specific release plan.

Site Name
Site Location
Persons/Organization responsible for release
Reasons site was chosen
Habitat characteristics, including foodplant, nectar sources, and basking sites
Threat amelioration
Existing disturbances

Degree of protection

Management plans

Size of site

Access (roads, trails, etc.)

Ownership and protection

Landscape context of site

Surrounding habitat map

Location of nearest extant *E. editha quino* populations

Location of nearby historical *E. editha quino* populations

Potential changes in land use surrounding site

Release design

Life stage

Number and source stock

Release procedures

Monitoring plan

Site management and restoration

Long and short term management and restoration goals

Planned management techniques

Evaluation program for management and restoration

4.6 Timeframe for Release and Management

The naturally dynamic nature of a metapopulation should be reflected in the timing of release and management for Quino checkerspot. Unlike species with slow growth rates and stable populations, the rapid fluctuations and shifting occupation of this species suggest an approach that is expeditious. Within the constraints described below for genetic management and disease, the benefits outweigh the risks for implementing a number of releases to attempt to reestablish (or restabilize) metapopulations. The release program will in essence be replicating a boom year for the species, where propagules from exploding populations are spread widely across the landscape (and propagules are not exhibiting directed dispersal to the highest quality sites), as occurred around Oak Mountain in 2010.

Therefore, all acceptable target sites (e.g. restored) within the current range should be evaluated for feasibility of release with less time and effort allocated for prioritization of those sites. Some general guidelines for desirable locations for release are articulated below, but the general approach should be biased toward moving forward with releases quickly.

5 Genetic and Demographic Considerations

The two primary criteria to go forward with a butterfly reintroduction effort are that (1) the number of individuals taken from the donor population will not affect the donor population viability and (2) the number of individuals will be sufficient for a viable reintroduced population. Demographic and population dynamic studies form the basis for achieving these two criterial. However, advances in genetic data collection and analysis make genetic approaches desirable as companion tools to inform reintroduction efforts.

5.1 Choice of Founders for New Populations

The choice of founders should rely on the careful consideration of multiple factors. Local adaptation can have important impacts on individual populations, for example, resulting in differences in host plant use or climatic response between populations (Aardema et al. 2011) and individual variation in phenotype and genotype can potentially alter reintroduction outcomes (Aardema et al. 2011; Wheat 2010), although we argue that this is unlikely to be a significant issue for Quino checkerspot. Also, maintenance of animals in captivity can result in unintentional selection and genetic shifts (Ford 2002; Lewis & Thomas 2001; Schultz et al. 2008; Snyder et al. 1996). Reintroduction efforts will therefore benefit from information available regarding founder quality with respect to the target reintroduction site but it should also be emphasized that inaction due to the absence of such information is more likely to cause further population extirpation and decline (Aardema et al. 2011).

Sources for reintroduction should be evaluated based on matches in morphology, environment, life history and potentially genetics to the target reintroduction site. This will minimize potential adverse impacts due to local adaptation differences. Similarly, the time in captivity for captive sources should not be too long (i.e. two years). The source should also be determined as robust for uses as a reintroduction source (i.e. adults observed each year, peak count exceeds 20). Therefore the donor population should be reasonably large and stable such that removal of individuals will not appreciably impact population dynamics. The resulting transfer of individuals should decrease extinction risk (Crone et al. 2007).

Knowing the structure and relationships of the target populations (both donors and recipients) can be important for successful conservation of the species through reintroduction. Ideally, genetics should be used to reveal both pre- and post- reintroduction population dynamics but lack of funding or expertise to pursue this goal should not delay reintroduction efforts. Genetic analyses of *Euphydryas editha* phylogeography using mtDNA and allozyme data have revealed conflicting patterns of genetic differentiation between populations and further markers are required to resolve *E. editha* phylogeographic questions (Saccheri et al. 2004). For example, the isolation and development of *E. editha* microsatellite markers (Mikheyev et al. 2010) could allow less costly but effective investigations into *E. editha quino* genetic structure. Genetic information can also be obtained in butterflies using non-invasive techniques that do not significantly affect survival of sampled individuals (Hamm et al. 2010). Applying these general principals from *Euphydras* species to Quino, it is not immediately obvious that there would be a high level of local adaptation. The species used to have a vast and interconnected range (Mattoni et al. 1997), with irruptive boom years that saw movement of individuals significant distances across the landscape (Murphy & White 1984). Even differences in host plant use across the range do not necessarily correlate with meaningful genetic variation. Singer et al. (1989; 2008) have reported other *Euphydryas editha* subspecies and populations exhibiting foodplant plasticity depending on their availability, so use of alternative foodplants may not necessarily represent a meaningful evolutionary shift (but see Singer & McBride 2010).

The importance of genetic variation across the range of Quino checkerspot could be investigated. If pursued, however, it should be guided by an *a priori* consensus among conservation geneticists of what level of variation would be biologically (as opposed to statistically) significant. Any potential risks of reintroduction from genetic stock that was not identical to that found in the past (e.g., in Orange County where no remnant populations exist), should be weighed against the demographic risk of extinction for the species. If subtle regional genetic variation is detected within the extant populations, and this variation can be reasonably interpreted as biologically relevant, this information should inform the choice of donor sites for reintroductions, and might lower the priority of reintroduction to some sites where genetically appropriate material is not available. Given the historic ecology and distribution of the species, however, this seems to be an unlikely scenario. In the absence of a full genetic analysis of extant populations, risk from genetic mismatch can be minimized by selecting donor sites that are nearest to and most ecologically similar to release sites.

5.2 Life Stage and Number of Founders for New Populations

A review of British butterfly reintroductions concludes that a release of a moderate number of adult butterflies (25–50) can successfully establish a population (Oates & Warren 1990). They suggest that best results will be obtained from releasing "freshly emerged *and mated* females" (emphasis added; Oates & Warren 1990). In a recent review of British and American butterfly propagation and release programs, Shultz et al. (2008) report that British reintroductions and augmentations predominantly use adult butterflies, while American efforts have used several life stages. Use of adult females a few days old for introductions is documented to be a successful technique in lycaenids (Marttila et al. 1997). The baton blue butterfly (*Pseudoeuphilotes baton schiffermuelleri*) was translocated several hundred miles by capturing gravid females in the field, confining them in darkened boxes for travel, and releasing at a recipient site (Marttila et al. 1997). Holdren and Ehrlich (1981) introduced *Euphydryas gillettii* to a site south of its existing range by taping 83 egg masses with 10,000 eggs on leaves of foodplants and releasing cups full of newly hatched larvae at one site and releasing 17 gravid females at a second site. Both approaches were successful, at least in the short term.

It is possible to establish butterfly populations by setting out larvae, pupae, or adults (Oates and Warren 1990, Schultz et al. 2008). Each approach has its benefits and depending on the source of the material, more than one life stage might be used. From a monitoring perspective, use of pupae or adults has advantages for release to a site presumed to be unoccupied because subsequent observation of larvae could be taken as confirmation of reproduction.

5.3 Appropriate Range

Appropriate release site targets and species range should be determined by careful examination of historical records and observations combined with genetic information where available. Recent local extinctions provide clear starting points where reintroduced populations are likely to become established relative to populations that were extirpated long ago. Nevertheless, under the appropriate circumstances historical locations should be considered if conditions are ideal for successful reintroduction (e.g. high quality/restored habitat, close proximity to populations). Museum records can define the appropriate range of *E. editha quino* and these are mapped in Mattoni et al. (1997) and Preston et al. (2012).

The FWS currently defines occupancy as contiguous habitat within 1 km of an observation of the butterfly. When two observations are within 2 km and there is connecting habitat, they are considered part of the same "occurrence complex." Because some populations are small, and time may be great between boom years, sites that are near and even connected by habitat with existing observation sites should be carefully evaluated as possible reintroduction sites and it should not be assumed that natural colonization will occur. Rather, release in these instances should be seen as assisted colonization and use stock from the occupied area nearby. Such translocations should be given high priority as a means to facilitate metapopulation dynamics.

6 Ecological Considerations

The primary reason for failure of butterfly reintroduction (or one can presume augmentation) efforts is low quality habitat at the recipient site (Schultz et al. 2008). Indeed this has been the experience in Great Britain (Oates 1992; Pullin 1996). Restored sites are frequently of lower habitat quality than donor sites for reintroductions (Chan & Packer 2006), so attention to habitat quality is of critical concern to reintroduction or augmentation success.

Habitat conditions should be assessed and compared against known requirements for a species before a reintroduction is implemented (Armstrong & Seddon 2008; Chan & Packer 2006). Ideally, the conditions that have caused extirpation or species decline should have been identified and corrected (IUCN 1998). The causes of decline/ extirpation for Quino checkerspot populations are varied and site-specific but include invasive grasses, urbanization, agricultural expansion, fragmentation and land management practices (Preston et al. 2012). The standards for deciding what habitat features are adequate for reintroduction are usually obtained from reference sites where the species is abundant (Chan & Packer 2006). Research establishing such minimum habitat requirements for Quino checkerspot has not been done, but Longcore et al. (2003) developed an envirogram that identified the key elements that are in the "centrum" (*sensu* Andrewartha and Birch 1984) of factors that determine population dynamics. These include resources, malentities (aka threats), availability of mates, and predators/parasites (Longcore et al. 2003). Similarly, connectivity should be evaluated at the landscape level in the larger metapopulation context.

6.1 Resources

6.1.1 **Space**

Resources needed to constitute a habitat patch for Quino checkerspot include a patch of physical space, the size of which (in addition to its quality) will determine how long a population upon it might persist. A small patch of <0.25 km^2 might support a small population for a number of years but would be susceptible to extinction within the order of years if not nearby other patches. 0.25–4 km^2 might be stable on the order of decades and incorporate several smaller patches. Larger areas (>4 km^2) that include nested networks of smaller patches should be stable over longer periods. If a captive breeding program is being maintained for purposes of a refugium, release into even small patches should be considered because these can become part of regional metapopulation dynamics and the marginal cost of providing material (either from the captive population or through translocation) is minimal.

6.1.2 **Microtopography**

A high quality habitat would have a diverse microtopography that provides areas of sun and shade and water availability. This is important to be able to provide foodplant resources under varying weather conditions (Weiss et al. 1988). For example, during drought conditions shady north-facing aspects may be preferred. The quality of larval foodplant and nectar sources will vary over time and space across microtopographic gradients, as will the distribution of different species of these plants.

6.1.3 **Nectar Sources**

Access to high-quality nectar enhances the reproductive output of adult butterflies and a range of native wildflowers are used for this purpose (Emmel & Emmel 1973; Mattoni et al. 1997; Murphy & White 1984; Orsak 1978). Quino have short tongues and prefer platform type flowers (M. Singer pers comm.). Flowers in the Asteraceae and Lamiaceae families as well as *Eriogonum* spp. and *Allium* spp. have been identified as well used. Although these need not be adjacent to larval foodplants, and Quino checkerspots may move several hundred meters for nectar sources (C. Parmesan, pers. obs.; also recorded for other subspecies, Gilbert & Singer 1973), proximity and abundance of nectar sources is important for reintroduction.

6.1.4 **Larval Hostplants**

The most important factor for most Quino checkerspot populations is the density of larval host plants. To our knowledge no calculations are available in the peer-reviewed literature that quantify the exact amount of hostplant necessary to support a small population, but it is likely to be on the order of thousands of plants. Multiple host plant species have of course been identified, which can play primary and secondary roles in supporting larvae. At a minimum, a release site should have thousands of primary host plants in a relatively constrained area (0.25 km^2).

6.2 Malentities

Malentities are those non-trophic interactions that result in direct mortality of individuals. Trampling, collection, road kill, and incidental mortality from grazing or severe weather events would all qualify as malentitites (Longcore et al. 2003). A suitable release site would by nature of its management and land use minimize these threats. Drought and fire could be conceived of as malentities, but fire can also contribute to the maintenance of core resources. Too frequent fire, however, should be avoided.

6.3 Mates

Access to mates is a limiting factor for low-density butterfly populations, especially for those species that are protandrous (Calabrese and Fagan 2004). It is likely that Quino checkerspot is also vulnerable to this problem. For a release program, this issue can be avoided by releasing mated females if adults are being released, or releasing larger numbers of larvae or pupae if these life stages are chosen. Mated females can immediately begin laying eggs without waiting to be mated. For subsequent years the size and quality of the habitat patch will determine number of mates available.

6.4 Predators, Parasites, and Disease

Relatively little is known about predation on Quino checkerspots by vertebrates or about the role of disease in limiting wild populations. Invertebrate predators, such as exotic earwigs and ants, are potentially limiting for eggs or early instar larvae (Pratt 1999). Potential release sites should therefore be managed to exclude or reduce abundance of exotic invertebrates. Other considerations regarding disease are discussed below in the context of captive propagation.

7 Technical Considerations

7.1 Captive Rearing

Pratt and colleagues have developed rearing and breeding techniques for Quino checkerspot that have been sufficient to maintain captive populations over generations (Pratt 2004, 2005, 2007, undated; Pratt & Emmel 1998; Pratt et al. 2000; Pratt & Stouthamer 2002). The sections below summarize the methods from these sources and sets out some guidelines for genetic line tracking and recordkeeping.

7.1.1 **Adults and Eggs**

Pratt has shown considerable success with hand-pairing adult butterflies. Males are aged 3 days before mating. The female is held by the wings and the tip of her abdomen rubbed gently against the antennae of the male. If the male is receptive he twists his abdomen and begins wing fanning. The female is then left with the male, with mating between thirty minutes and twelve hours generally being successful. Females are then set up in quart size containers with screening and an oviposition substrate consisting of one of the foodplants (Pratt et al. 2000). Eggs can be collected from the oviposition chamber regularly by replacing the foodplant so that time to hatch can be measured. Adult butterflies are fed a honey:water solution to provide essential amino acids and energy.

7.1.2 **Larvae and Diapause**

Once larvae hatch from eggs after 10–14 days, the early instar larvae are kept in containers with foodplant that are opened twice (to regulate humidity) and cleaned once daily (Pratt et al. 2000). These small larvae spin a silken shelter for protection. After growth through a few instars the larvae enter diapause, at which point they are put in a dark, dry room in "vials" (Pratt et al. 2000). This happens in the spring as the foodplant dries out.

Diapause can be broken in the spring by exposing the larvae to a small container with foodplant and moist paper towels (Pratt et al. 2000). Once broken and feeding vigorously (after about 10 days), larvae are transferred to flats of *Plantago erecta* with full-spectrum light and 75 watt incandescent set to 13 L: 11 D photoperiod. Pratt starts these flats of *Plantago erecta* each week starting in October to ensure a constant source of food through the growing season. Larvae apparently prefer young plants (see Pratt undated).

Postdiapause larvae are raised 50–150 in a tank with a flat of *Plantago* and cut branches of *Penstemon*. After 3–4 weeks the larvae pupate on the plants and are removed and stored individually. Pupae eclose after 10–14 days.

7.1.3 **Methods to Reduce Risk**

We recognize that the specific techniques that are used by any particular laboratory conducting captive propagation for endangered species will be different. This is to be expected. Some common features must be taken into consideration to minimize risks from any captive propagation effort and to maximize the ability of managers to track progress and assess compliance with mitigation measures. Risks that can be minimized through captive propagation design and practices include: genetic contamination, disease/parasite contamination of captive stock, transfer of disease/parasites to new locations, and selection/genetic drift in captivity.

7.1.3.1 Genetic Contamination

Genetic contamination of a captive population would occur when the target species is unintentionally given access to a related species or subspecies or individuals of the same subspecies from another area that is maintained at, or occurs near a rearing facility. The converse of this would be the escape of individuals of the captive species that might interbreed with the local fauna. Both of these possibilities can be avoided by establishing rearing protocols with multiple layers of containment, both for butterflies and for foodplant (see e.g., Johnson et al. 2010). This might include the use of a screened container to keep a butterfly and larvae on the branch of a plant, which is kept inside a larger container with mesh sides and sleeves to allow access, which in turn is maintained inside a greenhouse or pop-up shade structure (Johnson et al. 2010). In this manner, three layers of containment separate the endangered butterfly or larva from and local species that might be in the area, yet air circulation and sunlight can still be obtained. In some instances, such as that described for the current Quino checkerspot rearing program, keeping the butterflies inside a building may be the preferred approach.

7.1.3.2 Disease

Lepidoptera are susceptible to a range of diseases caused by bacteria, viruses, fungi, microsporidia, and nematodes (Boucias & Pendland 2001; Tanada & Kaya 1993). Mattoni et al. (2003) documented Bt and microsporidian infection in a captive population of Palos Verdes blue butterfly. The endo-symbiont *Wolbachia* is also known to have subtle but important demographic effects on butterfly populations (Nice et al. 2009). Release of diseased individuals into wild populations is of concern if these individuals will reduce fitness of wild individuals (Crone et al. 2007). Consequently, Crone et al. (2007) report that butterfly propagation programs avoid release if disease is found in captivity (e.g., for Oregon silverspot) (see also Pearce-Kelly et al. 1998).

Introduction of disease (bacteria, viruses, and other pathogens) and parasites can be minimized by practicing good hygiene with captive stock, maintaining multiple layers of containment, minimizing time in captivity, and segregating butterflies from different regions (Crone et al. 2007; Cunningham 1996). Good hygiene includes basic protocols to minimize transport of bacteria and viruses, including:

- Hand washing before and after handling stock;
- Removal of shoes or cleaning shoes with 10% bleach when entering rearing areas;
- Immediate segregation of butterflies or larvae that appear to be infected or ill;
- Quarantine of larvae that were co-housed with larvae that exhibit signs of infection (see e.g., Mattoni et al. 2003);
- Seasonal sterilization of equipment and rearing chambers;
- Monitoring humidity to avoid production of mold.

Multiple layers of containment will exclude access of captive butterflies to free-flying wild butterflies at the rearing facility. Separating genetic lines from different region is also desirable, both for genetic management reasons and to reduce the possibility of disease transfer. Given the irruptive potential of Quino checkerspots during boom years, segregation by region (at the county-scale) is adequate, since any endemic diseases would likely have already saturated regional populations.

Risk of transfer of disease to new locations (e.g., a recipient site of a reintroduction) can be minimized by the measures described above, and by selecting stock for release that have no outward signs of infection in captivity and are from the nearest location that is ecologically similar.

7.1.3.3 Parasites

Similarly, parasite/parasitoid transfer can be minimized by proper containment of captive stock and choice of life stage for release. Parasitism by tachinid flies and wasps (e.g. *Pteromalus puparum*) occurs in Quino checkerspots (Mattoni et al. 1997) but not commonly relative to other butterflies (e.g., *Euphydryas phaeton* or *Melitaea cinxia*; Lei & Hanski 1997;

Stamp 1984). Though effects of parasitoids on *E. editha* populations are not thoroughly understood, they are not believed to be having strong or primary effects on population dynamics (van Nouhuys & Hanski 2004) though Moore (1989) found a high incidence of parasitism in some Sequoia National Park *E. editha* populations. Adults eclosed in captivity are unlikely to harbor either parasites or disease.

7.1.3.4 Genetic Drift/Selection

The possibility for genetic drift and/or selection while in captivity is an issue for any captive breeding program (Ford 2002; Lewis & Thomas 2001; Schultz et al. 2008; Snyder et al. 1996). It is possible, for example, that selective pressure results in genetic changes favoring compliant butterflies that respond to mating in captive environments at the expense of necessary behaviors for obtaining mates in the wild. Two measures should minimize this risk for Quino checkerspots. First, any genetic line that is kept in captivity should be refreshed by the introduction of new wild genes at least every two years. This might involve complete turnover of genelines every two years if ample wild individuals are available (which is currently done for the Lange's metalmark breeding program) or introduction of wild genes to an ongoing program through outcrossing or addition of wild stock to a captive program as has been done by Pratt for the current Quino rearing program. This allows for the explosive population growth that is possible in captivity to occur, yet avoids any potential long-term risk from prolonged propagation.

Managing genetic diversity requires that captive breeding programs maintain meticulous records of the lineages of individuals in the population. A studbook may take the form of a computer database, which details critical life cycle dates (year egg laid, year(s) in diapause), sex, parentage, source location, and identification numbers for each individual in the program. These data can provide important information for genetic management, and represent a standard component of a captive breeding program across all animal taxa.

7.2 Release Protocols

The existing literature on butterfly releases does not point to a single strategy that is most effective for establishing a new population. For purposes of documenting success, however, it would be most practical to release a life stage or stages that allows for confirmation of subsequent reproduction at the recipient site. Site-specific release plans should take this into consideration, which will probably mean release of post-diapause larvae, pupae, and/or adults. Subsequent observation of pre-diapuase larvae would confirm mating, oviposition, and hatch of offspring. If coming from a captive propagation program, pupae and adults are likely to minimize the probability of introducing disease. A standard approach, subject to revision by site-specific plans, would be release of 40–80 mated female butterflies soon after adults are observed flying at the nearest ecologically similar occupied habitat. Mated females are recommended if stock is coming from a captive population so that some eggs can be obtained to maintain a genetic line prior to release. It also means that females can be released directly onto foodplants and begin ovipositing without a delay for copulation. The number to be released can be scaled up based on the area of suitable habitat available. The relatively large number of butterflies recommended for release is to maximize the probability that eggs are laid in locations that are microclimatically favorable (Weiss et al. 1988), and likely provides far more genetic variation than occurs in natural colonization, where half of new populations are founded by a single female (Austin et al. 2010).

7.3 Post-Release Monitoring

Post-release monitoring at the release site should include a transect that surveys all of the habitat area (a full survey) or a systematic, repeatable sample of that area (Pollard 1977; Pollard et al. 1975). Such surveys, if they are exhaustive across the target habitat, can document spatial distribution of females after release (Mattoni et al. 2002). Adult surveys should be continued each year following the release following USFWS protocols for at least five years and estimates of abundance made from these data (Mattoni et al. 2001; Watt et al. 1977; Zonneveld et al. 2003). These data can also be used to track the geographic variation in site use by adult butterflies if converted to a grid across the site (see Longcore et al. 2010). Longer term monitoring is desirable and should be part of the management plan outlined in the site-specific release plan. Additional monitoring for post-diapause larvae or pre-diapause larvae could provide additional information about the status of the introduced population.

Some researchers are attempting to incorporate distance sampling and other techniques to measure butterfly detectability into transect surveys. Although some marginal improvement in accuracy could result from such efforts, the added effort is unlikely to provide insights beyond those already gained from transect surveys and geographic analysis of occupancy. Also abundance of endangered species like Quino is usually too low to parameterize distance-sampling estimators. Repeat surveys immediately after release could be cost-effective and useful to calibrate population estimates at a particular site.

8 Adaptive Management Structure

Propagation and release of endangered butterflies should be undertaken within the context of adaptive management (Blumstein 2007; Sneddon et al. 2007) and may incorporate structured decision making (Starfield & Bleloch 1991). An adaptive approach involves exploring alternative ways to meet management objectives, predicting the outcomes of alternatives based on the current state of knowledge, implementing one or more of these alternatives, monitoring to learn about the impacts of management actions, and then using the results to update knowledge and adjust management actions (Holling 1978; Lancia et al. 1996; Walters 1986). The steps of the adaptive management process, as described by Haney and Power (1996) are as follows: (1) compile, inventory, and exchange information, (2) state goals and objectives, (3) develop model of system in question, (4) identify and implement management action, (5) monitor, (6) analyze data and evaluate model (see also Longcore et al. 2007). Central to this approach is explicit identification of stakeholders and the decision-making team.

For the current situation with Quino checkerspot, the species recovery plan (U.S. Fish and Wildlife Service 2003), 5 year review (U.S. Fish and Wildlife Service 2009b), and this captive propagation and release plan serve to compile, inventory, and exchange information about the species, in addition to the many unpublished reports and scientific papers that have been produced since listing of the species. The recovery plan and 5 year status review set goals for the reintroduction of the species into certain areas and this plan articulates a further goal of reintroduction into restored habitats that might be naturally colonized over time. Importantly, pre- and post-release decisions and monitoring should be considered on landscape and metapopulation scales as well. Each site-specific release plan will need to articulate objectives and should express a model of the butterfly-foodplant-landscape system into which the butterfly is to be released. This will be a particularly important step, as each reintroduction/release effort will constitute an experiment that can help understand the species' habitat requirements. After release and monitoring, these a priori predictions of habitat suitability (which would provide estimates of area, foodplant density, nectar resources, topographic context, etc.) can be tested with data on butterfly persistence. If conceived in this manner, managers will be able to contribute to a growing cumulative knowledge base, while still taking action with potentially significant conservation benefits.

8.1 Decision analysis framework

This adaptive management approach can be implemented through following a structured decision making framework. This framework provides a series of steps at which the Fish and Wildlife Service will make a decision to proceed with any given release project. We recommend the establishment of a rearing and release steering committee that is made up of experts in butterfly propagation and release across the United States. These experts should have experience with captive rearing and release, but not be actively involved in Quino checkerspot conservation so that their review can constitute an arm's-length independent assessment.

8.1.1 **Establish need for release**

Releases should be considered for any habitat within the historic range where adequate biotic and abiotic resources can be provided to support a small population (i.e. habitat management is in place which diminishes the threats which caused original population decline or extirpation). This plan hypothesizes that the minimum area is 0.25 km^2 with thousands of larval foodplants. As part of a release-supported metapopulation, even small populations can contribute to long-term species viability. Plans should only go forward if the project would contribute to species recovery and be undertaken with the intent of establishing a population that will self-sustain for a period of years if not decades. Priority should be given, however, to plans to introduce butterflies into unoccupied habitats that are likely to support the species indefinitely.

8.1.2 **Rapid overall assessment**

Program managers should consider the legal, socioeconomic, ecological, technical, and regulatory requirements that must be met for the project. Only projects with adequate support, resources and expertise should proceed. If local support, resources or expertise are not adequate, managers should identify potential collaborators to have in place before proceeding.

8.1.3 **Identify multidisciplinary team**

All projects require an interdisciplinary team to accomplish the various tasks, and this team should be in place before project planning continues. Team members must have the expertise and qualifications to undertake the various aspects of the project, including legal planning, habitat restoration and management, acquisition of release stock, any captive breeding or propagation, release and post-release fieldwork and monitoring.

8.1.4 **Conduct habitat assessment**

A formal habitat assessment should be conducted that quantifies the status and distribution of resources such as foodplants, nectar sources, topographic heterogeneity, and other potential habitat elements. This assessment will be used to develop the release plan and as a means to test hypotheses about habitat suitability and success of release efforts. A quantitative habitat assessment will be essential for each release to contribute a data point in the understanding of habitat needs. Critically, the habitat assessment must have some historical and management context such that assurances can be made that release into the target site will not result in immediate extirpation due to continued and unmitigated threat persistence (e.g. invasive species).

8.1.5 **Identify donor population**

The next step in deciding if a release program should go forward is whether an appropriate donor population is available. The default recommendation is use of the nearest population from an ecological similar location. Removal of individuals from this population must, however, be deemed to have no impact on that population. Any refugia populations can be considered as sources for releases, but only if they consist of genelines that have not been held in captivity for an extended period (> 2 generations).

8.1.6 **Identify any needed rearing or breeding resources**

Unless a direct translocation is envisioned, resources are likely to be needed to either propagate butterflies from the donor site (e.g., rearing from egg to adult) or even breeding to increase the stock available for release. Resources for these efforts should be identified and secured before proceeding.

8.1.7 **Assess socioeconomic and legal requirements**

Before proceeding further, the legal, financial, and socioeconomic considerations must be considered and any potential roadblocks identified. Release of an endangered species to a location not recently occupied requires federal authorization and is much more likely to be successful if it has the support of municipal and state government as well. Any potential impact on surrounding non-target areas should be identified, such as movement of the species on to non-protected land adjacent to a release site.

8.1.8 **Write site-specific release plan and secure administrative permissions**

If all of the foregoing steps have been completed, a formal site-specific release plan should be prepared, following the outline presented above. This plan should be the basis for securing all administrative permissions, including review under the National Environmental Protection Act, and presents the managers' conceptual model of the butterfly-habitat system to be restored.

8.1.9 **Evaluate release-stock for suitability**

With a release plan in place and permissions secured, the existing stock for release should be evaluated again. If a translocation is proposed, the donor site should be identified in the release plan. If captive stock is to be used, their number, health, and genetic lineage should be considered to make a final assessment on their suitability for release. The decision to move forward with the release should be flexible in response to either the captive stock or a donor population and only go forward when the considerations enumerated above have been addressed.

8.1.10 **Transport and release**

Transport to the release site should be planned to minimize stress on the life stage being used. For adult butterflies, transport in a cooler or at night might be appropriate to minimize thermal stress. Animals should be transported with at least two layers of containment to avoid loss of the butterfly or accidental introduction should one of those layers fail. Release protocols will depend on the life stage used. For adult butterflies, release should be done when flight conditions are warm so that butterflies are not easily predated. For larvae, a usual approach would be to interleave the foodplant from the laboratory with foodplant in the field. This also should be done in warm conditions so that larvae can quickly move to safe conditions. Any scheme to set out pupae should include a means to protect the pupae until they eclose, either through a protective cage or keeping them in containers where butterflies can emerge but predators would be largely excluded.

8.1.11 **Post-release documentation and monitoring**

The usefulness of the release program as an adaptive management effort will depend on each release being documented thoroughly and there being follow-up monitoring to ascertain the status of the released individuals and their progeny. The development of the release plan should result in the conditions surrounding each release being documented and each individual release should be coupled with a report that confirms that the plan was followed and identifies where deviations from it were made.

Post-release surveys can take many forms and their number and intensity will depend on resources available. At a minimum, protocol-level adult butterfly surveys should be conducted at the site each year following the release. This could be done immediately following release of adults (within days) to map behavior and distribution within the new habitat. Flight surveys during subsequent years will provide the basic information of whether butterflies are present and reproducing.

Regular transect surveys can be used to develop estimates of population size in the event that releases result in successful colonization and reproduction. Notwithstanding the problems with population estimates that lack detectability estimators (Haddad et al. 2008; Nowicki et al. 2008), population estimates based on transect counts, and even peak transect count numbers have been shown to provide reliable information (Collier et al. 2008;

Haddad et al. 2008; Isaac et al. 2011; Mattoni et al. 2001). Despite the suggestion that distance sampling be incorporated into butterfly transect counts, this has not proven feasible for endangered species because of the low number of butterflies observed is usually inadequate to parameterize the detectability estimates.

Larval surveys could be illustrative the first year after release if only adult butterflies were released, in which case presence of larvae would confirm overwintering of the offspring of the colonists. Larval surveys could be used in successive years as well.

8.2 Release objectives

Current Service documents prioritize release of quino checkerspots for the purpose of reintroducing a population in Orange County (U.S. Fish and Wildlife Service 2009a). This should be pursued, given the availability of suitable habitat and the loss of Orange County to the distribution of the subspecies. The goal of any release program should be broader, and seek to establish Quino in whatever restored and protected habitats can support it and might not be currently occupied. Although many such releases might not result in lasting populations of the species, they would put butterflies in place to be able to take advantage of optimal weather conditions when the might occur. This would require changes to the Recovery Plan, which recommends that reintroduction should be initiated only when an entire occurrence complex or population distribution has not been occupied for 3 consecutive years. With the increasing size of the occurrence complexes, this is not a reasonable provision and occupancy elsewhere around a suitable but unoccupied potential release site should not stand in the way of a release.

The historic distribution of Quino checkerspot populations extended throughout much of Los Angeles, Orange, San Bernardino, Riverside, and San Diego counties (Mattoni et al. 1997). Reintroduction efforts should remain within the borders of these counties and use multiple historical species presence records and baselines as guides for reconstructing its historic range (Bonebrake et al. 2010) (see Figure 1).

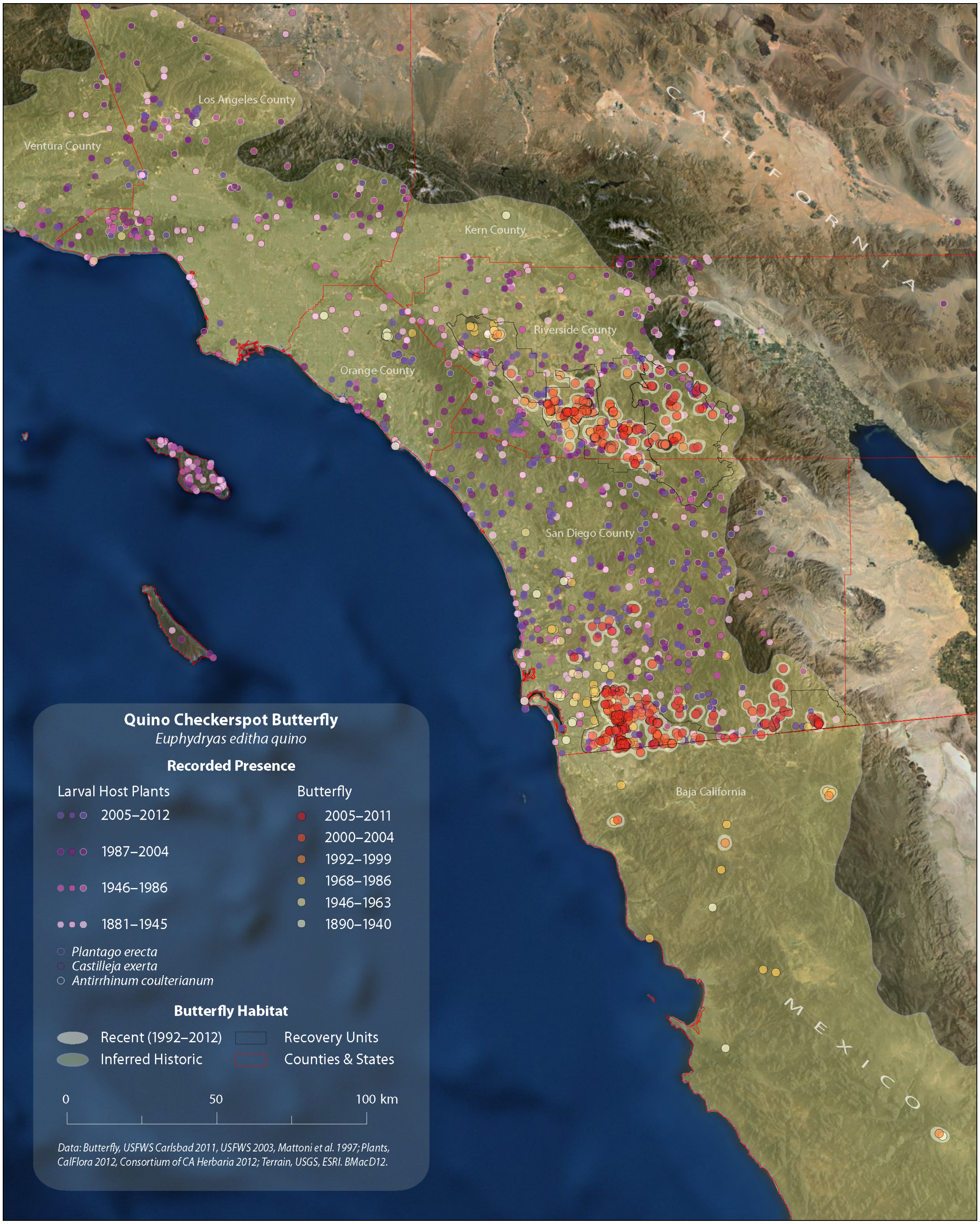

Figure 1. Presumed historic range of Quino checkerspot, with historic and current observation locations from FWS and foodplant distribution from herbarium records.

8.3 Assessment and endpoints

A comprehensive analysis centered on demographics could inform when to cease reintroduction or augmentation efforts (Schaub et al. 2008). When it is determined that a population is self-sustaining reintroduction efforts should be scaled back with continued periodic monitoring to ensure that the population remains viable. As defined in the recovery plan, success (defined as "resilience") in any occurrence complex achieved if any decrease in the number of occupied habitat patches over a 10- to 20-year period is followed by increases of equal or greater magnitude (U.S. Fish and Wildlife Service 2003).

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